

The Botanical Review

Interpreting Botanical Progress

Founded and published by

H. A. GLEASON AND E. H. FULLING

Managed and edited at The New York Botanical Garden by

E. H. FULLING

Volume III

1937

Published Monthly at
Lime and Green Streets, Lancaster, Pa.

The Botanical Review

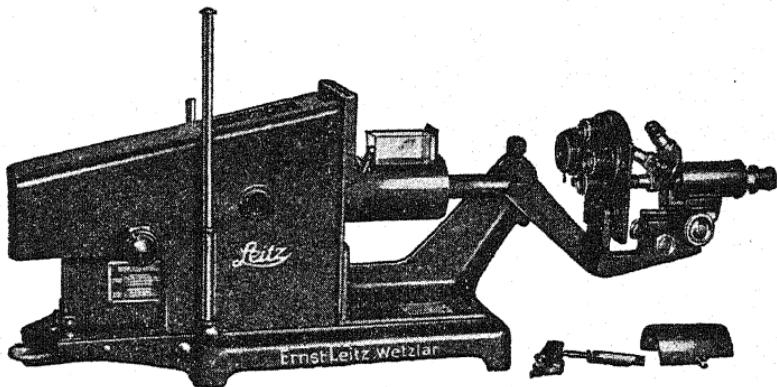
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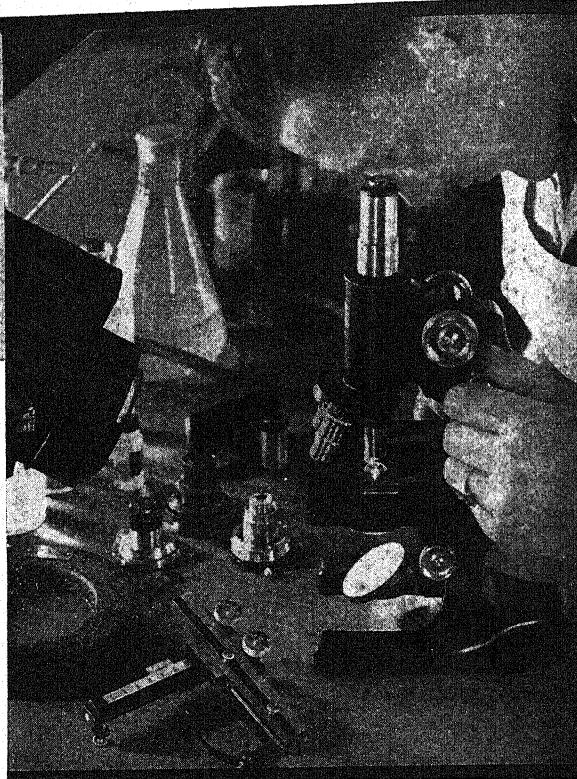
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THE BOTANICAL REVIEW

VOL. III

JANUARY, 1937

No. 1

THE PLANT VACUOLE

CONWAY ZIRKLE

*Morris Arboretum and Department of Botany,
University of Pennsylvania*

The first vacuoles to be noticed by microscopists were not the large, seemingly empty regions in fully differentiated plant cells, but the smaller pulsating bodies which can easily be seen in many protozoa. These latter, which we call "contractile vacuoles," were described by Spallanzani as early as 1776 and, although he misinterpreted their function and assumed that they were respiratory organs, his description was remarkably precise and accurate. "It (the vacuolar organ) consists of two stars, with a very minute globe in the center, and situated, as one may say, in the foci of elliptical animalcula of the largest or middle size. Whether the animalcule moves or not, the stars are always in alternate or regular motion. Every three or four seconds the minute central globules swell like a bladder to three or four times their natural size and then fall; the inflation and efflation are performed very slowly. The same is done by the rays of the stars, except that inflation of the globe empties the rays, and inflation of the rays empties the globules." These organs were also described by many of the protozoologists who followed Spallanzani and they were the bodies to which the term "vacuole" was first applied (Dujardin, 1841).

The characteristic vacuole of the mature plant cell is so structureless and so passive that it failed to attract the attention of the eighteenth century biologists, and when it was finally noticed it was described merely as a part of the cell, distinct from the dense, granular cytoplasm whose streaming seemed so spectacular. Cytoplasmic streaming had been discovered by Corti (1774, 1776), and the discovery substantiated by Fontana (1776), but it was promptly forgotten until 1810 when it was rediscovered by Treviranus. Amæi (1818, 1824), Agardh (1826), Meyer (1827, 1835, 1838), Slack (1834), Dutrochet (1837) and Schleiden (1842) investigated

the phenomenon in detail, and Meyen (1835, 1838), in his illustrations of the cytoplasmic stream, delineated the vacuoles very clearly. Schleiden (1842) recorded the physical differences between the streaming cytoplasm and the cell sap as follows: "In most plants in the families of the Characeae, Najadaceae and Hydrocharitaceae, there is observable in each cell a simple current ascending on one side and descending on the other, the fluid constituting which differs in color, consistence (mucosity) and insolubility in aqueous fluids, from the remainder of the transparent cell-juice." In the ends of certain desmid cells, (Naegeli (1849), Cohn (1854), DeBary (1858) and Fischer (1884) described the specialized vacuoles which contained crystals of gypsum and Charles Darwin (1875), and later Gardiner (1885) and deVries (1886), recorded the fragmentation of the colored vacuoles in the tentacles of *Drosera rotundifolia*, changes which accompanied the movements of the tentacles of this insectivorous plant. Went (1888) reached the general conclusion that all plant cells were vacuolate and Hof (1898) showed clearly that there were vacuoles even in the cells of root meristem, where the cytoplasm was densest.

The use which could be made of vacuoles in the investigation of osmosis seems to have been realized first by deVries (1877, 1884) who pointed out the fact that the cytoplasmic layer in the larger plant cells apparently acted as a perfectly semi-permeable membrane. The rôle of turgor in maintaining the shape and rigidity of plant tissue had long been known and Dutrochet (1828) had pointed out the connection between the osmotic pressure in the petioles of sensitive plants and their reaction to stimuli. Plasmolysis had been described by Pringsheim (1854), Naegeli (1855) and others, but it was not until the eighth decade of the last century that a thorough investigation of the osmotic properties of plant cells was undertaken (deVries, 1877, 1884; Hamburger, 1883; and others). Today vacuoles are of interest to biologists chiefly because of their importance in the investigation of osmosis and permeability. The enormous literature in this field has been summarized adequately by Jacobs (1924, 1935) and Osterhout (1931, 1936) and, accordingly, it need not be reviewed here.

Recently, an attempt has been made to homologize the plant vacuole with the Golgi-apparatus of the animal cell and a number of investigators have utilized the more modern cytological methods

in an effort to answer the questions which have been raised. Reviews of this cytological literature have been published by Dangeard (1923), Guilliermond (1929, 1930, 1933), Kechoski (1932) and Weier (1933) and the reader is referred to these papers for a more complete bibliography of the subject.

ORIGIN OF VACUOLES

The first investigators who described vacuoles were not particularly interested in their origin. There seems to have been a general though tacit assumption that they appeared *de novo* as the cytoplasm accumulated enough water to form visible droplets. Undifferentiated or meristematic cells were held to be non-vacuolate and the vacuoles were thought to develop as concomitants of cell enlargement and tissue differentiation. DeVries (1885), however, challenged this generally accepted view and argued that vacuoles were developed from distinct primordia which he called *tonoplasts*. These tonoplasts were supposedly small plastid-like bodies which occurred in meristematic cells and which multiplied only through the division of pre-existing tonoplasts. As they absorbed water they enlarged and became vacuoles, the tonoplast itself developing into the vacuolar membrane. Van Tieghem (1889) accepted DeVries' conclusions but renamed the tonoplasts *hydroleucites*. Went (1888) did not endorse the view that vacuoles were derived from "massive" tonoplasts but held that they were derived from pre-existing vacuoles, and when he demonstrated that not only the reproductive cells of algae but also meristematic cells of the phanerogams were vacuolate he reached the broad general conclusion that "all plant cells contain vacuoles."

Unfortunately the methods used by DeVries and Went in demonstrating the presence of vacuoles were open to criticism. Their technique for rendering vacuoles distinct was to place cells in a 10% to 15% solution of potassium nitrate. As Klebs (1890) emphatically stated, cells so treated were not normal. Furthermore, Went's demonstration of vacuoles in cells of marine algae by placing the plants in distilled water was unsound. For this reason his observations on living untreated cells were ignored and when Pfeffer (1890) experimentally caused vacuoles to arise *de novo* in the plasmodium of a Myxomycete by introducing into it soluble granules of asparagin, Went's conclusions were generally held to

be untenable and the older view of Hofmeister prevailed, *i.e.*, that vacuoles appeared first in those cells which enlarged through the imbibition of water. Thus meristems are figured and described as non-vacuolate in most botanical text-books in spite of Hof's (1898) excellent demonstration to the contrary.

In 1910 Bensley found canals in the meristematic cells in the roots of *Allium*, *Lilium* and *Iris* which seemed to be identical with those found by Holmgren (1902) in liver cells and in epithelial cells of the suprarenal gland. Bensley showed that these canals were perfectly normal vacuoles which increased in size and ultimately fused to form the large central vacuoles of the fully differentiated plant cells. Other plant cytologists had missed seeing these canals because they had examined only those specimens which had been fixed in such a manner that all the cytoplasmic organization had been destroyed.

Recently, attention has been focussed again upon the origin and development of vacuoles by the work of Guilliermond (1914) and the Dangeards (1916, 1917, 1918, 1923). Guilliermond (1914, 1923, 1929, 1930, 1933) noted small colored vacuoles in the immature petals of a number of flowers. These vacuoles were of the size and shape of mitochondria and indeed were first looked upon as mitochondria by both Guilliermond and Dangeard, but the former, following Pensa (1917), demonstrated conclusively that they were very different from mitochondria in their chemical properties. These colored vacuoles were not highly specialized structures differing from the vacuoles in the primary meristem, but they were typical in every way except for their pigment. The emphasis placed upon them by Guilliermond and Dangeard was due to the fact that they were easier to see in living untreated cells than were the uncolored vacuoles in root tips and stem growing points.

Bailey (1930), working with living tissue, has shown that the initials of the cambium are vacuolated. Indeed, he reported certain cambial cells to be as highly vacuolated as plant hairs. He found a striking seasonal variation in the size, shape and number of vacuoles, the total volume of vacuolar material being greatest during the period of rapid cell division. In tissues produced from the secondary meristem, the vacuoles are obviously not derived from minute viscous mitochondria-like bodies. Actually, the vacu-

oles in the secondary meristem have no general form nor are they derived from any characteristic primordia. They may be rod-shaped, thread-like or spherical. They may fuse into a single large central vacuole or fragment into many separate globules which later may become beaded chains or tangled skeins. In many cells, the vacuoles are carried along in the streaming cytoplasm and the form they assume is conditioned by the cytoplasmic activity.

The vacuoles in the primary meristem are essentially like those in cambium (Zirkle 1932). All of the cells are vacuolate and the shape and size of the vacuoles seem to be conditioned primarily by the activity of the cytoplasm and the size of the cell. When the cytoplasm is quiescent, they tend to become spherical in the smaller cells; when it streams actively, they either become rod-like or are drawn out into Holmgren canals, or they may even form a reticular apparatus. Thus the shape of the vacuoles is of little importance in itself, for it merely indicates the activity of the cytoplasm. As a rule, the larger the meristematic cell, the greater the relative amount of vacuolar material, and in the exceptionally large apical cell in the root of *Osmunda* they may be as large as they are in the cambium of *Pinus* during the growing season.

There is no evidence that vacuoles in either the primary or secondary meristem develop from tonoplasts, hydroleucites, mitochondria or any other cytoplasmic inclusion and, while the smaller vacuoles may often assume the forms ascribed to these primordia, no useful purpose is served by using any of these terms. All meristematic cells which have been inspected adequately contain vacuoles (Hof, 1898) which are constantly changing their shape, fusing and again fragmenting. The only mode of origin that could be discovered for any individual vacuole was the division of a pre-existing vacuole, as reported by Went (1888). (Guilliermond (1923) has stated that vacuoles originate *de novo* in *Saccharomyces* and *Saprolegnia*.) The fact that this has not been observed in any meristematic cells does not mean, of course, that it never occurs there, for it is quite possible that a new vacuole could be mistaken for a fragment of a dividing one in a cell whose contents are in rapid motion. The fact that new vacuoles can be made artificially (Klebs 1890, Pfeffer 1890, Mollendorf 1936, *et al.*) has no real bearing on this problem as it does not justify the inference that such vacuoles occur in nature.

THE PLANT VACUOLE AND THE ANIMAL GOLGI APPARATUS

When Bensley (1910) found that the canals described by Holmgren in cells of liver and kidney epithelium occurred also in root meristems, he suggested that these canals represented the Golgi apparatus in both animals and plants. As it was a very simple matter to demonstrate that these Holmgren canals developed into the large central vacuole of the mature plant cell and, even, that they themselves were smaller vacuoles drawn out into a canalicular form by the streaming cytoplasm, he inferred that the plant vacuole was the homologue of the animal Golgi apparatus. Guilliermond and Mangenot (1922), Dangeard (1923), Parat and Painlevé (1924, 1925), Guilliermond (1927), Scott (1929), Bose (1931) and others have accumulated a great deal of evidence to support this view. On the other hand, Bowen (1926, 1927, 1928, 1929), Gatenby (1929) and Beams and King (1934, 1935) hold that vacuoles and the Golgi apparatus are separate structures and that the Golgi material in the plant cell constitutes the "osmophilic platelets," a distinct cell organ. Weier (1932, 1933), after studying Bowen's preparations, reported that the Golgi zone is similar in many respects to plastids.

In spite of their differences of opinion as to what constitutes the Golgi apparatus in plants, these investigators really agree in all of their essential findings. There is little argument as to questions of fact and little question but that some of their inferences are reared on an insufficient factual basis. As Bowen (1926, 1927) started the controversy, it would be well to summarize his conclusions briefly and to cite the evidence upon which they are based. He stated that he was entering the field of plant cytology without any particular bias as to the possibilities in prospect and that he would attempt to apply to plant cells those methods which had long been familiar to animal cytology. At the outset he discarded Golgi's original technique of *reducing* silver in a reticular apparatus on the ground that silver nitrate will "*impregnate*" almost anything on occasion. He relied primarily for his identification of the Golgi material upon its ability to reduce osmium tetroxide when the osmium was protected by such oxidizing agents as chromic acid or potassium bichromate, although he recognized that plant vacuoles which contained tannin would also reduce the osmium. Bowen concluded that there was a special Golgi material, that the reticulum

was but one of a number of forms that it assumed and that the material was of a fatty nature. Specific tests for fats generally failed to give positive results, but Bowen cited two instances where Golgi material contained fatty substances (Weiner 1926, Cowdry 1911). He concluded that "So far as fat tests have succeeded, they indicate the presence of Golgi substances as a material reality." The plant vacuole could not be composed of the Golgi material because the vacuole contents were "never lipoidal but always watery."¹ The real homologue of the Golgi apparatus in plants was the *osmophilic platelets*. Gatenby (1929) is in essential agreement with Bowen while Beams and King (1935), by means of the ultra-centrifuge, have shown that the osmophilic platelets in the onion root are distinct from the vacuole and that their relative specific gravity is comparable with that of the Golgi apparatus.

The alternate interpretation of the plant Golgi apparatus is based on a number of resemblances between the Golgi apparatus of animals and the plant vacuole both as to size and shape, and reaction to cytological reagents. Ten years before Golgi (1898) reduced silver in the reticular apparatus and thus discovered the structure which bears his name, Bokorny (1888) reduced silver in the vacuoles of *Spirogyra* from a very weak solution (1: 100,000) of ammoniacal silver nitrate. Three years previous to this, deVries (1885) reduced osmium in plant vacuoles from a 1% solution of osmium tetroxide in 10% potassium nitrate. It is interesting to note here that the essentials of the two most usual methods for demonstrating the Golgi apparatus in animal cells were first used to "stain" the plant vacuole. Bokorny interpreted the reaction as indicating the presence of an active (reducing?) protein in the cell sap. Klemm (1892) objected to this interpretation, however, and rightly pointed out that before the presence of this active protein could be demonstrated in the cell sap it would first be necessary to prove that the vacuole contained no tannin or other reducing agent. DeVries considered the reduction of osmium to be due to tannin. He also discovered that vacuoles were fixed with salts of other heavy metals, $HgCl_2$, $AgNO_3$, $CuSO_4$, etc. Loew and Bokorny (1877) showed

¹ Vacuoles are essentially aqueous although there is evidence that they contain a finely dispersed partially hydrated lipid (Scarth, 1926). Bailey and Zirkle (1931) and Lison (1935) show how it is possible to explain the virage of vacuoles stained with vital dyes by assuming that they contain traces of a finely diffused fatty component.

that the content of the vacuoles was precipitated in a granular form by bases. The usual cytological preparations fixed by acids showed the vacuoles as hollow spaces.

It seems remarkable that Holmgren canals were not demonstrated in plant cells until 1910. The delay was undoubtedly due to the fact that plant meristems were investigated only in specimens fixed with reagents which, while preserving a high degree of nuclear detail, disorganized the cytoplasm and destroyed its finer structure. It was not until a different type of fixation was tried that an accurate picture of the cytoplasm and vacuoles was obtained and the resemblance between the vacuoles in plant meristems and the reticular apparatus of animal cells was demonstrated. These more modern fixing fluids depend for their characteristic images either upon formaldehyde or upon some bichromate in a solution on the basic side of pH 4.8-5.2. A fatty acid in the mixture destroys the image (Zirkle 1933). Thus Hof, Holmgren and Bensley were able to investigate accurately the size and shape of vacuoles but only as "negatives," for vacuoles, which contain no tannin, appear merely as vacant regions surrounded by cytoplasm. On the other hand, when tannin is present, the vacuole can be preserved and colored by the salts of any heavy metal. Iron is particularly useful, as Dangeard (1923) and Zirkle (1932) have pointed out.

Obviously, the identification of the Golgi apparatus in the plant cell is largely a matter of definition. Just what is meant by the Golgi apparatus? We really know very little about it; by comparison, our knowledge of vacuoles is extensive and precise. Vacuoles have long been recognized as perfectly normal parts of living plant cells. Their behavior in living tissue and reaction to vital stains have been described by a number of workers (Guilliermond, Dangeard, Irwin, Bailey, etc.); their contents in certain coenocytic forms have been analyzed by numerous investigators (Wodehouse 1917, Crozier 1919, Brooks 1922, Osterhout 1922, Hoagland and Davis 1923, Irwin 1923, etc.). On the other hand, the reticular apparatus discovered by Golgi has been definitely recognized only in tissue which has been subjected to prolonged and complicated chemical treatments (Cowdry 1924). Parat and Painlevé (1925) have indeed found a reticular structure in living animal cells which stains with Neutral Red and it is quite possible that this reticulum contains reducing substances which react with silver and osmium

to form the Golgi apparatus. Neutral Red, however, is no certain test for a vacuole, for it also stains certain cytoplasmic granules while it does not collect in vacuoles more basic than pH 6. The Golgi technique depends primarily upon the reduction within the cell of some heavy metal—generally silver or osmium. Chemically these tests are so nonspecific that it is impossible to make any precise inference as to the nature of the Golgi material. Any vacuole which contained a chloride—and most vacuoles contain chlorides—would retain the silver. A vacuole which contained tannin would be blackened by both silver and osmium. Osmium would be reduced also by oil droplets, oleoplasts, fatty acids, etc. Indeed, Ludford (1924) defined the Golgi apparatus as ". . . that region in the cytoplasm which brings about the reduction of osmium tetroxide at a lower temperature or in a shorter time than is required to produce a total blackening of the cell." As far as we know at present, the Golgi material or the osmophilic platelets may be composed of any one of a number of substances or a mixture of many substances. And the material that reacts to the Golgi technique in one cell may be very different from the material that reacts to the technique in another cell. We have no right at present to speak of a specific Golgi material. Golgi himself described a "reticular apparatus," but later work has shown that the material in this apparatus need not always have a reticular form. We should remember, moreover, that the only "reticular apparatus" found thus far in the plant cell is the vacuole drawn out by the streaming cytoplasm. It is obvious that before we may homologize the Golgi apparatus with any known structure in the plant cell, we will have to define the term much more precisely than we have thus far succeeded in doing.

CONTRACTILE VACUOLES

Both the regular pulsating vacuoles and those which discharge at irregular intervals are most easily observed in the protozoa and, consequently, their structural development and their function have been studied most extensively in this group. Indeed, the very existence of such vacuoles in plants has often been overlooked, although Lloyd (1928) has shown that they are to be found in the colored flagellate *Euglena*, in the zoospores of many algae and in the Myxomycetes. A very specialized type occurs in those vege-

tative cells of *Spirogyra* which are developing into gametes (Lloyd 1926, 1928). As the cells contract, previous to their passage into the conjugating tubes, special contractile vacuoles form and empty the cell-sap from the large central vacuole into the surrounding medium.

SALT CONTENTS OF VACUOLES

The contents of vacuoles can be analyzed directly only in such favorable material as the marine coenocytic alga, *Valonia*, and the aquatic group, the Characeae. *Valonia* is a spherical green alga composed of but a single multinucleate cell which often attains a diameter of 5 cm. The large central vacuole in the mature plant sometimes contains more than 25 cc. of cell sap, enough to be analyzed accurately. The cylindrical internodal cells of *Chara* and *Nitella* frequently attain a diameter of 1 mm. and sometimes reach an extreme length of 15 cm. The vacuolar contents of these cells can also be analyzed and the findings are exceptionally important, although we must be careful not to assume that all plant vacuoles contain what are found in these extremely specialized cells.

As early as 1847 Naegeli stated that the cell of *Valonia* was filled with water which, on tasting, seemed to be even saltier than sea water. Famintzin (1860) also described the vacuole as filled with salt water. Meyer (1891) made a careful analysis of the cell sap and compared it with sea water. He found the vacuoles to contain sodium, potassium, calcium and magnesium; chlorides, sulphates, nitrates and phosphates, and he was astonished to note that they contained much more potassium and much less sodium than sea water. More recent analyses have been made by Wodehouse (1917), Crozier (1919) and Osterhout (1922). The following table comparing the electrolytes in the cell sap of *Valonia* with Bermuda sea water is from Osterhout (1922):

Element	Sea water, parts per thousand	Cell sap, parts per thousand
Chlorine	19.6	21.2
Sodium	10.9	2.1
Potassium5	20.1
Calcium45	.7
Magnesium	1.31	trace
SO ₄	3.3	.005

Brooks (1922), Irwin (1923) and Hoagland and Davis (1923) have analyzed the cell sap of *Nitella*. The following table is from the analysis of Hoagland and Davis:

Analysis of Nitella Sap and Pond Water

Element	Sap, parts per M.	Pond water, parts per M.	Factor of concentration
Sodium	230.	5.	46
Potassium	2120.	trace	?
Calcium	410.	31.	13
Magnesium	430.	41.	10
Chlorine	3220.	32.	100
SO ₄	800.	31.	26
PO ₄	350.	.4	870

A glance at these two tables shows that certain electrolytes penetrate the semi-permeable layer of cytoplasm and accumulate in the vacuoles *against an osmotic gradient* until they are far more concentrated within the cell than they are in the surrounding medium. Attempts to explain this behavior led to the present intensive investigation of permeability, one of the most complicated problems in all physiology. For a clear discussion of this problem the reader is referred to Jacobs (1924, 1935) and Osterhout (1928, 1936). It will be sufficient here to suggest two simple explanations. First, the penetrating substances may be combined chemically with something in the vacuole, forming a compound which cannot diffuse out from the cell; second, that some condition of the vacuole, such as its pH, alters the electrolytes from a relatively penetrating form to a relatively non-penetrating form. These explanations will be discussed briefly later.

Our knowledge of the contents of typical plant vacuoles which are too small to be analyzed directly is much more fragmentary. Undoubtedly, free oxalic acid exists in the vacuoles of many species, for Kerr (1933) has shown that it is in vacuoles of the root hairs of *Limnobium*, and Chester and Whittaker (1933) have found it in the expressed juice of a number of plants. Indeed, the higher plants can be divided into two groups: those whose expressed sap contains oxalic acid, and those whose expressed sap contains calcium chloride. When extracts from these two groups are brought together there is a white precipitate of calcium oxalate, often copious

enough to be mistaken for a precipitation reaction of the plant proteins (Chester and Whittaker).

SPECIFIC GRAVITY AND OSMOTIC VALUE OF VACUOLES

Mottier (1899) centrifuged cells from a number of different plants; from various algae, from leaves of mosses, *Elodea*, *Valisneria*; from the staminal hairs of *Tradescantia*; from trichomes of *Urtica*, *Cucurbita*, etc.; from the seedlings of *Zea*, *Vicia*, *Ricinus*, etc. He found that the colorless, fully hydrated vacuoles were lighter than the cytoplasm and were displaced toward the centripetal end of the cell. Milovidov (1930) substantiated the discovery of Mottier but found, in addition, that the smaller vacuoles of rose petals which contained anthocyanin were heavier than cytoplasm and were displaced centrifugally, as were the smaller vacuoles in the root tips of *Hordeum*. Beams and King (1935), by means of the air-driven ultra-centrifuge, obtained a force 400,000 times gravity and were thus able to place the various cell organs in separate layers. They found the vacuoles in the root tips of *Phaseolus* to be lighter than the cytoplasm and osmophillic platelets but heavier than the lipoid-like material and the fat globules. Incidentally, this seems to furnish the best proof to date that the osmophillic platelets are not composed of the simple fatty substance assumed by Bowen to be the Golgi material, as their specific gravity is greater than that of the fully hydrated vacuoles.

The osmotic value of the cell sap has been investigated by two different methods, both of which are inaccurate to some extent. The first, initiated by deVries (1884), consists of placing specimens in fluids of known osmotic strength and recording the osmotic value of the solution when incipient plasmolysis occurs. Such solutions are supposedly isotonic with the cell sap. Fitting (1916), Hanning (1912), Ilgin (1915), Ursprung and Blum (1916), Beck (1928), Weber (1929) and many others have used this method. Recently, Ernest (1935) has shown that this method does not yield exact results because of the time required for the plasmolysis to show. In fact, where the time lag is great, the plasmolysis may be due to injury. Approximate values may be obtained by this method, however, and she reports that the osmotic pressures of the cell sap in several species of *Iris*, *Saxifraga* and *Isatis* seem to

lie somewhere between that of a .4M and .5M solution of cane sugar or somewhere between 9 and 11 atmospheres.

The second method consists in pressing out the sap from the plant to be investigated and measuring its osmotic value directly. Such fluids, of course, contain cytoplasm, intercellular fluids, water from the xylem ducts, stored sugar, etc., and, consequently, their osmotic values may differ greatly from those of the vacuoles. The tremendous range in the osmotic value of the juices extracted from different plants, however, indicated that there is also a great variation in the osmotic values of different vacuoles, for it would be unreasonable to assume that there is no correlation whatever between the expressed juice and the vacuolar sap.

The osmotic value of the expressed juices of aquatics, as a rule, is much less than that of land plants. In *Elodea* it may be as low as 5 atmospheres. In the rain forests of Jamaica, Harris and Lawrence (1917) found it to range between 8 and 9 atmospheres in the herbs and between 11 and 12 atmospheres in the woody plants. In the leaves of mangrove trees, growing in salt-water swamps, it varied between 25 and 34 atmospheres. In certain plants from the Great Salt Lake region the expressed juice had the astounding osmotic value of 153 atmospheres (Harris, Gortner, *et al.*, 1921). Sen-Gupta (1935) has measured the osmotic value of the sap of a number of plants in India and finds that it ranges from 3 to 50 atmospheres. An excellent review of this subject is to be found in Miller (1931).

pH OF THE CELL SAP

Expressed plant juices are acid as a rule and are alkaline only rarely and then only slightly so. These juices, of course, are not composed merely of cell sap, although the acids found in them have frequently been located in the cell vacuoles. It is interesting to note that when tissue is crushed the juice which is first given off contains a much lower concentration of electrolytes than that obtained when greater pressure is applied so as to crush more of the cells. The reader is referred to the papers of Haas (1916, 1917), Atkins (1922), Smith and Quirk (1926). The following table is compiled from the data of Smith and Quirk. The fact is worth recording that the leaf of *Begonia lucerna*, which heads the table,

is the most acid tissue on record, for the juices range from pH .9 to pH 1.36, approximately as acid as .1 N sulphuric acid:

Plant	pH of Juice Expressed from Leaves
<i>Begonia lucerna</i>	0.9 - 1.36
<i>Oxalis</i> sp.	1.70 - 1.72
<i>Rumex crispus</i>	3.53 - 3.56
" <i>obtusifolia</i>	3.64 - 3.67
<i>Agave</i> sp.	4.30 - 4.31
<i>Allium cepa</i>	4.33 - 4.45
<i>Ananas</i> sp.	4.72 - 4.74
<i>Musa</i> sp.	4.74 - 5.04
<i>Nephrolepis exaltata</i>	5.20 - 5.22
<i>Polystichum lonchitis</i>	5.32 - 5.35
<i>Colocasia antiquorum</i>	5.33 -
<i>Tradescantia zebrina</i>	5.44 - 5.47

The chief acid involved is oxalic.

The pH of the cell sap can be measured directly only with great difficulty and the values obtained are generally somewhat inaccurate. The acidity of the food vacuoles of certain protozoa has also been measured (Shapiro 1927, Howland 1928), together with the changes in pH which accompany the digestion of their inclusions. From a point approximating neutral (pH 6.6 - 7) they become as acid as pH 4.3 during digestion and then become more basic (pH 5.4 - 5.6) as digestion is completed. Particularly favorable plant material, where the pH of the vacuoles may be determined readily, is to be found in flower petals, colored berries, red cabbage leaves, etc., where the cells contain anthocyanin pigments dissolved in the cell sap. The color of these pigments varies with changes of acidity and, if the pigment is known, it is possible to obtain the pH of any solution in which it is dissolved by comparing the solution with a color standard. Indeed, these pigments were used as indicators of acidity by Boyle as early as 1664 when he employed an extract from violets. Wray (1670), Lister (1671) and Grew (1682) also described color changes which occurred when various plant pigments or flower petals were subjected to acids and bases, and the practical value of these pigments as indicators was soon established by the chemists. Schwarz (1892) correlated the change from red to blue during the anthesis of flowers of *Pulmonaria*, *Anchusa* and *Lathyrus* with a decrease of acidity,

and Willstätter (1914) noted that the same pigment was responsible for the color of both the rose and the cornflower, and assigned a value of pH 5.5 to the cell sap of the former and of pH 7.2 to the latter. Haas (1916) investigated the pH of a number of plant cells by means of these natural indicators. He extracted the indicators and recorded their color in buffers of known hydrogen ion concentration. By matching the buffered solution of these anthocyanin pigments with the pigments in the vacuoles of the living cells, he was able to obtain the pH of the cell sap. He found that the vacuoles ranged from pH 3 to pH 7 or even 8, depending upon the species investigated. Irwin (1919) and Brooks (1926) used these anthocyanin pigments to measure changes in the acidity of cell sap which occurred during physiological experiments. Buxton and Darbshire (1929) and Smith (1933) showed that there were a great many different anthocyanins, each with a characteristic color range. Smith recorded the pH of the cell sap in the petals of 25 species of flowering plants and found that it ranged from pH 3.1 to pH 7.8.

The hydrogen ion concentration in vacuoles which contain no natural indicators is more difficult to estimate. The vacuolar contents of *Valonia* and *Nitella* can be extracted in quantities sufficient to measure although when we consider the difficulty in maintaining the CO₂ tension we would expect a relatively large experimental error. Indeed, Crozier (1919) found the cell sap of *Valonia* to range from pH 5 to pH 6.7 in different specimens, the average being about pH 6. On the other hand, Hoagland and Davis (1923) reported that the cell sap of *Nitella* remained nearly constant at pH 5.2, even when the surrounding medium varied from pH 5 to 9. The pH value of the smaller microscopic vacuoles of course cannot be obtained by this method.

The fact that a number of basic dyes, which are pH indicators, will penetrate the cytoplasmic layer and accumulate in the vacuoles has led a number of investigators to estimate the pH of the vacuoles by their virage when stained. There are many sources of error in this method as the dyes in question have relatively large salt and protein errors and, in addition, an error called to our attention by Dangeard (1916) caused by a substance in the vacuoles named "metachromatin." Different vacuoles, sometimes within the same cell, may acquire very different colors when stained by such dyes

as Neutral Red (Dangeard 1916, Bailey and Zirkle 1931). Living cells in sections cut through cambium, phloem and medullary rays of a number of gymnosperms contain two categories of vacuoles (Bailey and Zirkle 1931), designated "A" type and "B" type for convenience. The "A" type vacuole contained tannin and was stained magenta with Neutral Red, the "B" type contained no tannin and was stained orange. The tannin-containing vacuoles were stained with 36 different basic dyes and their virage indicated that their hydrogen ion concentration was very close to pH 4.4. The "metachromatic" error did not enter into this measurement.

On the other hand, the range indicator method gave very uncertain results with the "B" type vacuoles. Twelve dyes were found which stained these vacuoles, and the color of the stained vacuole matched different buffered solutions of the dyes ranging from pH 7.2, when they were stained with Neutral Red, to pH 13, when they were stained with Brilliant Cresyl Blue, depending upon the particular dye that was used. Obviously, such results show that the vacuoles contain some substance which markedly alters the normal virage of basic dyes. If chloroform is shaken with the various buffered solutions, the dyes will be partitioned between the chloroform and the buffer when the two layers separate. When the buffer is as acid as pH 4.4, the apparent pH of the "A" type vacuole, the color of the dye is always the same in both layers. With more alkaline solutions, however, the color of the dye in the chloroform layer may be very different from its color in the buffer itself. With all of the twelve dyes employed, the color of the stained "B" type vacuole matched the color of the dye in the chloroform which was in contact with the solution buffered at pH 5.8. These results can be explained if we assume that the "B" type vacuoles contain some substance which alter the virage of the dyes in a manner comparable to the alteration caused by chloroform and that the pH of the vacuoles is approximately 5.8. This point will be discussed at greater length under the heading "metachromatin." Lison (1935) has also called attention to the fact that the virage of the basic dyes which penetrate the cytoplasmic layer is conditioned by this "metachromatic" factor and that the previous records of alkaline vacuoles based upon the virage of these dyes are in error.

The sulphonated dyes used as indicators by Clark and Lubs are insoluble in chloroform and ether and are not subject to the "metachromatic" error. These dyes, however, do not penetrate the cytoplasmic layer as do the basic dyes, but they can be inserted into the vacuoles directly by the modern technique of micro-injection and once in the vacuoles they are unable to diffuse outward. Chambers and Kerr (1932) have injected them into the living root hairs of *Limnobium*, and find that the vacuoles there have a pH of $5.2 \pm .2$.

METACHROMATIN AND THE COLLOIDAL CONTENTS OF VACUOLES

The idea that vacuoles contain metachromatin can be traced to the early conception that they originated from the hydration of relatively dense colloidal globules. These globules were labelled metachromatin because of their reaction to vital dyes. Like bodies had been reported in bacteria (Babes 1889, 1895) and in yeast (Guilliermond 1902). In the latter the globules fused to form a larger more hydrated body which had the property of concentrating the usual vacuolar stains. The investigations of both Guilliermond and the Dangeards showed that the vacuoles of the higher plants contained some substance which affected their color when they were stained with vital dyes and which ultimately united with the dye to form precipitates. The existence of some such substance has been amply confirmed by the subsequent work of Mangenot (1929), Dufrenoy (1929), Bailey and Zirkle (1931), Lison (1935) and others.

The colloidal nature of certain highly specialized vacuoles in storage tissue was recognized as soon as it was shown that they developed into aleurone grains. Aleurone grains had been seen and described by Hartig (1855, 1856), Halle (1858) and Maschke (1859), while Pfeffer (1872) showed that they were composed essentially of proteins. It was first thought, however, that these grains were developed from a form of plastid that occurred in such tissue as the endosperm of *Ricinus* and in a number of other seeds. Maschke located the grains in vacuoles and Gris (1864) and Wacker (1888) showed that the grains themselves were partially dehydrated vacuoles. Guilliermond (1907) and Dangeard (1923) especially have traced the cytologic development of these grains in great detail, and the evidence seems conclusive that reserve protein is stored in vacuoles which, consequently, are colloidal.

There is also good evidence that other types of vacuoles contain colloids. Perhaps all vacuoles are more or less colloidal although many contain but slight traces of organic compounds. Bokorny (1888) held that vacuoles contained protein, but on very insufficient evidence. Weber (1925) and Frey (1926) assumed the presence of colloids to explain the observed viscosity of the vacuolar contents, the latter measuring the rate at which the gypsum crystals settled downward in the end vacuoles of desmids. Bailey (1930) described the formation of Liesegang rings when certain "B" type vacuoles were stained with Neutral Red and, as these vacuoles contained no flavones or other such substance, the presumption is that the included colloid may be of a type that is very widely distributed in the vegetable realm. Indeed, many of our observations would be explained very simply if we assume that "metachromatin" itself consists of finely dispersed colloidal particles of some fatty substance. Its fatty composition would explain its effect upon the virage of vital stains as its characteristic color effects have been shown to resemble those of chloroform (Bailey and Zirkle 1931) and of ether (Lison 1935). Such a substance evenly dispersed in an aqueous medium would probably be in colloidal particles. Scarth (1926) has shown that the colloidal contents of certain vacuoles behave as partially hydrated lipoids.

TANNIN

Tannin was definitely identified in cell sap by Wigand (1862) and Wiesner (1862) when they treated the cells with iron salts and with alkalies. With the former the sap turned blue, green or black depending upon the species tested and the particular part of the plant from which the specimen was taken; with the latter the sap became orange or yellow. The tannins were first investigated by a number of French and English chemists between 1790 and 1800, and the subsequent publications on the subject are far too numerous to be cited in a review of vacuoles, although many of the studies of tannin are cytological. The reader is referred only to some of the more recent work, that of Wisseling (1910, 1914), Lloyd (1922), Guillermond and Dangeard in numerous papers and of Mangenot (1927, 1929).

That tannin is widely distributed throughout the vegetable kingdom was shown by Bailey and Zirkle (1931) who report its occur-

rence in 272 representatives of 42 orders and 90 families of the Pteridophyta, Gymnospermae and Angiospermae. It is found especially in the phloem, in the mesophyll of leaves, in fruit and insect galls, mixed with pyrogallol, and in the peripheral regions of young roots. Sometimes the vacuoles contain only traces of tannin, as in certain cells in the root tips and young leaves of *Pinus*. When such tissue is fixed with fluids containing heavy metals, precipitates form about the rims of the smaller vacuoles and they appeared as hollow spheres, circles or platelets, with "osmophilic" peripheries and "osmophobic" centers.

TONOPLASTS

The membrane which surrounds a vacuole is called a tonoplast and although cytologists are not in agreement as to its structure or composition or even as to whether it is part of the vacuole itself or of the cytoplasm, there is little doubt but that the vacuole is surrounded by a layer which is impervious to many of its components. The name tonoplast was introduced, as has been stated, by deVries (1884) who looked upon it as a plastid-like vesicle which grew by hydration into a vacuole. Van Tieghem (1888), who accepted the tonoplast theory temporarily, and Went (1888) collected much evidence in favor of deVries' hypothesis but most botanists discarded it, so today the word tonoplast is merely the name given the membrane which surrounds the vacuole and the use of the term does not imply the acceptance of any theory of vacuole origin.

Recently, the tonoplast has been investigated by a number of physiologists. Lloyd and Scarth (1926) have shown that it has many of the physical properties of lecithin and that some of its characteristic activities are not dependent upon the life of the cell. Indeed, many investigators have isolated tonoplasts with their enclosed vacuoles, and find that they maintain many of their typical characters even when floating free from the cell (Bailey 1930, Chambers and Hofler 1931, Plowe 1931, Eichberger 1934, Lederer 1935). On the other hand, tonoplasts are undoubtedly a part of the cytoplasm in living plant cells as they can be observed to flow as a part of the cytoplasmic stream. Indeed, Weber even considers the evidence of an inner membrane of the cell as incomplete (See Weber 1932, Hofler 1932). Obviously, there is no need to con-

sider the tonoplast as a definite membrane differing in chemical composition from the rest of the cytoplasm. Its very contact with the cell sap would give it properties not possessed by the remaining cytoplasm. Whatever its origin or composition, it acts as a membrane impervious to many substances, and it thus resembles the plasma membrane, although it differs in certain respects for Osterhout, Damon and Jacques (1927) have shown that the inner and outer surfaces of cells differ.

Micromanipulative investigations have shown that the vacuolar membrane is tough, elastic and sticky (Chambers and Hofler 1931, Plowe 1931, Eichberger and Lederer 1934). Mothes (1933) holds that it is extremely thin, homogeneous, elastic and non-miscible with water or cytoplasm. It contains an essential albuminous component, although he found no evidence of a lipoid constituent. The permeability of the tonoplast will be discussed later.

THE VITAL STAINING OF VACUOLES

Strictly speaking, it is perhaps inaccurate to describe vacuoles as ever being really stained. They are certainly not stained in the sense that chromosomes, cell walls or textiles are stained. They merely become colored through their ability to concentrate weak solutions of basic dyes, just as oil droplets concentrate Sudan IV from dilute alcoholic solutions. A "stained" vacuole is thus essentially a colored solution, although frequently the basic dye which is absorbed unites chemically with certain of the acids dissolved in the cell sap. This ability of vacuoles to absorb and accumulate dyes and other electrolytes against an osmotic gradient is one of their most interesting properties and it has stimulated some of the most fundamental physiological research in recent years.

As has been stated, two explanations suggest themselves. The first is that the cell-penetrating electrolytes combine with something in the vacuole to form a non-diffusible compound. The electrolytes would then be trapped within the vacuole until all of the hypothetical components were used up, when, of course, equilibrium would be reached and no more dye would accumulate. Scarth (1926) reports that in certain cases the penetrating dyes combine with colloidal material in the cell sap. This first explanation certainly accounts for the observed behavior of the "A" type or tannin-containing vacuoles. Basic dyes which penetrate the

cytoplasmic layer color these vacuoles brilliantly, forming precipitates as staining progresses—often until the vacuoles are nearly filled—whereupon the remaining cell sap loses its color (Bailey 1930, Bailey and Zirkle 1931). This explanation is inadequate, however, when applied to the "B" type vacuoles, which contain no tannin, although perhaps basic dyes combine with some vacuolar substances in all vital staining.

Before proceeding with the discussion it will be well to emphasize a factor in the problem of permeability which has generally been overlooked, and, in consequence, a number of unnecessary errors have appeared in many of the earlier contributions. We must remember that before a dilute solution of a dye can "stain" a vacuole, it must both penetrate the cytoplasmic layer and accumulate in the vacuole itself. These two processes occur at the same rate, of course, only when none of the penetrating dye diffuses out from the cell. If the dye leaves the cell as rapidly as it enters, the vacuole will not be colored, for a solution of the dye in the vacuole as dilute as it is in the exterior solution will show no color upon microscopic examination. Thus the failure of a dye to stain a vacuole is no evidence of the dye's inability to penetrate the cytoplasmic layer. Much of the criticism of Overton's classical hypothesis, that substances soluble in both water and fats penetrate the cell with relative rapidity, has been based on the alleged inability of certain fat-soluble dyes to enter the cell. Thus Rhodamine (vital) has been cited as a lipoid-soluble dye which penetrates living cells extremely slowly when it really accumulates in vacuoles, which can trap it with tannic acid, as rapidly as any other dye that has been investigated (Bailey and Zirkle 1931). Likewise, the cells of *Nitella* have been reported as impervious to Methyl Red regardless of the pH at which staining was attempted. This dye does not accumulate in "B" type vacuoles (the type of *Nitella* vacuole) but it penetrates the cytoplasmic layer very rapidly and accumulates in "A" type vacuoles. Kerr reports that it does not stain the vacuole in the root hair of *Limnobium* (a "B" type vacuole) but that it accumulates very rapidly in such vacuoles when tannic acid is injected into them. In spite of Osterhout's warning (1916), the neglect of this very real distinction between rates of penetration and rates of accumulation has led to a somewhat oversimplified picture of the effects of pH upon the rates of penetration.

Apparently any basic dye which can penetrate the cytoplasmic layer will be trapped by a vacuole which contains tannic acid. Bailey and Zirkle (1931) found 35 basic dyes which stained the "A" type vacuole, but only 12 of these dyes accumulated in the "B" type, although they penetrated the cytoplasmic layer. On the other hand, acid dyes, which are insoluble in lipoids, apparently cannot enter the living plant cell, for no such dye was found to be permeable out of the 36 dyes tested. When such non-permeating dyes are injected into vacuoles (Chambers and Kerr 1932) they do not diffuse outward, as do the lipoid-soluble basic dyes, but remain in the cell until death occurs. They can neither get into nor out of living cells.

Each of the permeable dyes has a characteristic pH range at which it can enter the cell (Bailey and Zirkle 1931). Such dyes as Neutral Red, Brilliant Cresyl Blue, Methylene Blue, Auranin, Pyronin, etc., penetrate rapidly from basic solutions, slower from slightly acid solutions and not at all from buffers in the more acid ranges. On the other hand, Methyl Red, Ethyl Red, Brilliant Green, etc., penetrate more rapidly from acid solutions, slowly if at all from the more alkaline buffers. Rhodamine (vital) accumulates in the vacuoles at a uniform rate throughout the entire pH range at which the dyes were tested. Obviously, the assumption that dyes penetrate in the form of free bases will not explain all of the effects of the pH upon the rate of penetration of the dyes.

The second explanation of the accumulation of electrolytes in vacuoles assumes that some condition of the cell sap, such as its pH, alters the penetrating dye to a form which is less permeable. Such a dye would thereupon diffuse outward from the vacuole more slowly than if entered until it reached equilibrium at a relatively high concentration. Irwin (1923, 1925, 1926) measured very carefully the effects of pH upon the rates at which Brilliant Cresyl Blue accumulated in the vacuole of *Nitella*. She found that the dye accumulated rapidly from basic buffers, slower from neutral or slightly acid solutions, and not at all from solutions buffered at the pH of the cell sap. Thus the dye seemed to be trapped by the hydrogen-ion in the vacuole for from such solutions it could not enter the cytoplasmic layer. In agreement with this interpretation is the work of Bailey and Zirkle (1931) who reported that all of the 12 dyes, which they found to accumulate in the "B"

type vacuole, were dyes which entered the cell relatively rapidly from basic solutions but very slowly if at all from solutions at the pH of the vacuole itself. All of the 23 dyes which entered the cell but which did not accumulate in the "B" type vacuole penetrated rapidly from solutions buffered at the pH of this type of vacuole and thus they could not be held in the vacuole by a pH trap but could easily penetrate the cytoplasmic layer from the cell sap. It is very important for us to realize that electrolytes which accumulate in plant vacuoles are not necessarily those which penetrate more rapidly than those which do not accumulate there. As far as we know at present, those which accumulate may really penetrate more slowly and over a more restricted pH range. Further investigation will have to decide this question.

SUMMARY

Spallanzani described contractile vacuoles in animals as early as 1776. Meyer (1835) depicted the large central vacuole characteristic of the mature plant cell in his illustration of cytoplasmic streaming and Schleiden (1842) recorded the difference between the vacuolar contents and the cytoplasm itself. Charles Darwin (1875) first noted the fragmentation of the colored vacuoles which accompanied the movement of the tentacles of *Drosera*, and deVries (1877) called attention to the importance of vacuoles in the investigation of osmosis. Went (1888) reached the general conclusion that all plant cells contained vacuoles and Hof (1898) showed that even meristematic cells were vacuolate. Today vacuoles are of interest to biologists chiefly because of their importance in the study of permeability.

Plant vacuoles are derived from the division of pre-existing vacuoles. There is no definite proof at present, however, that they never originate *de novo*. Their size and shape in meristematic cells are determined primarily by the cytoplasmic activity. They may be either globular, rod-like, canalicular or tericulate. They show many resemblances to the animal Golgi apparatus, both as to their structure and their reaction to cytological reagents.

Vacuoles have the property of accumulating electrolytes against an osmotic gradient, both the inorganic salts necessary for plant nutrition and the basic dyes used for vital staining. Vacuoles, in general, have a specific gravity less than that of cytoplasm but

greater than that of the oil droplets in the cells. Their osmotic value in different species varies from about 3 to 150 atmospheres and their hydrogen ion concentration from pH .9 to pH 8.

In the endosperm of a number of seeds the vacuoles develop into aleurone grains. They contain much ergastic material. There is evidence that all vacuoles contain some colloidal material. Their staining reactions can best be explained by assuming that they contain some substance, metachromatin, that acts as a partially hydrated lipid. Many vacuoles contain tannin, flavones or anthocyanin pigments. Oxalic acid is also found in the vacuoles of a number of different plants.

The vacuolar membrane is called a tonoplast. It is a liquid membrane in many cells and circulates with the cytoplasmic stream. This membrane is semi-permeable, like the plasma membrane. Any investigation of the accumulation of substances within the vacuole must be concerned with their inability to penetrate the tonoplast from the vacuole as well as their ability to penetrate the cytoplasmic layer.

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TERTIARY FLORAS OF EASTERN NORTH AMERICA*

EDWARD W. BERRY
The Johns Hopkins University

INTRODUCTION

The greatest impediment to a botanical or zoological approach to geological history is the general lack of realization of the enormous lapse of time involved and, consequently, a complete lack of perspective or orientation. A treatise could be written on this subject but it will suffice to recall, by way of illustration, how John Lindley—an acute enough botanist and an outstanding figure in the history of botanical thought—was constrained because of this lack, and inspired by his hostility to evolutionary ideas, to see cacti, arborescent spurges and other systematically advanced types in the sigillarias and their associates of the Carboniferous period.

It is important, therefore, in any discussion of Tertiary floras, to furnish some sort of time-table as a frame of reference. The science of geology has not yet reached the position in which it is possible to correlate events very precisely from continent to continent, but the chronological terminology should, as far as possible, be international rather than provincial, and the majority of scientific nations are moving in this direction, if only to be understood, and despite the deplorable resurgence of nationalism in their points of view on all other questions.

The following abbreviated scheme will be sufficient for the present purpose. At the left are the familiar and understandable major divisions of the Tertiary. In the fifth column are the stages of the international time scale which make up the Tertiary, and at the right are the principal plant horizons thus far known in eastern North America. This serves to indicate how incomplete the paleobotanical record of the 54 to 63 millions of years of the whole Tertiary¹ really is in eastern North America.

With but a single exception, the known Tertiary floras of eastern North America are confined to the present day physiographic province known as the Coastal Plain, the Atlantic and

* So little is known of the floras of the Quarternary period in eastern North America that the present discussion is limited to the Tertiary period.

¹ Taken from Barrell's estimates based upon radioactivity. U. S. Geol. Survey Bull. 769: 5. 1925.

Quaternary Period—Pleistocene and Recent						
Cenozoic Era	Tertiary Period					
	Eocene		Oligocene	Miocene	Pliocene	
	Upper	Middle	Lower	Upper	Pontian stage	
					Sarmatian	
				Middle	Tortonian	
					Helvetician	
			Lower		Burdigalian	
					Aquitanian	
	Upper	Middle	Lower		Chattian	
					Rupelian	
					Lattorffian	Vicksburg plants
		Upper			Ludian	Jackson plants
					Bartonian	Claiborne plants
		Middle			Auversian	Wilcox plants
					Lutetian	
		Lower			Ypresian	
					Sparnacian	
					Thanetian	
					Montian	Midway

Gulf Coastal Plain, or as the Atlantic Coastal Plain—the last usage being preferable.

The Atlantic Coastal Plain, now submerged northeast of Long Island and partially emerged from New York to Mexico, is unique among similar features of the earth in its extent, in its lack of any considerable deformation, and in the relative continuity of its picture of geological history from the Lower Cretaceous to the present—a lapse of time variously estimated as being between 120 and 149 millions of years.¹ Its paleobotanical history is fairly continuous from about the middle of the Lower Cretaceous through the Upper Cretaceous and the Eocene. In other words, it gives a rather more complete picture of that part of the floral history of the world than does any other region during a time when the flowering plants (angiosperms) assumed the dominant rôle on land which they display at the present time.

The Coastal Plain lies to the south and east of the Appalachian region, which last has been a land area since before the close of the Paleozoic era. This old land, although probably not the original home, was at least one of the theaters of evolution of the flowering plants. From western Greenland to Texas there have been found abundant records of land plants from the Lower Cretaceous into the Tertiary, and in this region the flowering plants make their appearance toward the close of the Lower Cretaceous (Potomac group of formations) associated with many descendants of Jurassic ferns, cycads, and conifers. These flowering plants increase greatly in number and variety during the Upper Cretaceous, which time marks the first modernization of the floras of the world, but they are accompanied until the close of the Cretaceous by the dwindling representatives of the older Mesozoic floras.

In the latest Upper Cretaceous floras of this region, that of the Ripley formation, about 40 per cent of the genera are unknown in the earliest Eocene and over 20 per cent are entirely extinct. This clearly indicates that the old and rather widespread impression that with the appearance of a considerable number of angiosperms in the mid-Cretaceous, terrestrial floras were rapidly transformed from a Mesozoic to a Cenozoic facies, and that the "Age of Flowering Plants" started in mid-Cretaceous and antedated the "Age of Mammals" by the whole of the Upper Cretaceous period, is most uncritical. Dramatic statements like the foregoing quotations

sound well, but are no more exact than the "Age of Algae," or the "Age of Cycads," or any of the other "Ages" that embroider the text in popular scientific writing.

The second great modernization of terrestrial floras marks the dawn of the Cenozoic era—not that this time marked the sudden ringing up of the curtain on a new scene with new actors—there was no cataclysmal cause back of it. Nor was change sudden. Old types of plants had been gradually dropping out and new ones appearing either as a result of autochthonous evolution or by immigration from other centers of evolution, and it suddenly becomes apparent to our limited vision, especially if there is a considerable time interval between the latest Cretaceous and the earliest Eocene sediments, as is the case in the Atlantic Coastal Plain, that we are in a new floral world. In regions like the western interior of North America, or the Mediterranean region of Europe where sedimentation was more nearly continuous, it seems impossible to put one's finger on the boundary between the two periods or to differentiate the floras, and of course if there were complete sedimentary records anywhere, then boundaries, either chronological or organic, would not be discernible.

THE LOWER EOCENE

Aside from scattered and poorly preserved traces of terrestrial plants in the marine sediments of the earliest Eocene of this region, comprising less than a score of genera, the first extensive Tertiary flora is that of the lower Eocene, or Wilcox flora.

Wilcox is the group name for the post-Midway lower Eocene formations—the name coming from a locality in Alabama where they are typically developed and contain fairly distinctive marine faunas that afford a basis for differentiating four formations.²

The Wilcox flora comes from over 130 localities scattered from Alabama to the Rio Grande, developed most extensively along the shores of the Mississippi embayment, which at that time flooded the Mississippi valley northward to the mouth of the Ohio. Between five and six hundred species have been described in 180 genera, 82 families, and 43 orders.

² Berry, E. W. U. S. Geol. Survey, Prof. Paper 156. 1930.

The largest families, in the order of their magnitude, are:

Lauraceae	Sapotaceae	Apocynaceae
Caesalpiniaceae	Anardiaceae	Celastraceae
Moraceae	Myrtaceae	Polypodiaceae
Papilionaceae	Combretaceae	Arecaceae
Rhamnaceae	Juglandaceae	Rutaceae
Sapindaceae	Sterculiaceae	Meliaceae
Mimosaceae	Araliaceae	

Eighty-three of the genera make their first appearance in the geological record at this time.

This flora is largely coastal and indicates a warm temperate climate and an abundant rainfall, more tropical in its facies than that of the late Upper Cretaceous flora which preceded it in this same region. It shows great contrasts with the contemporaneous floras of the western interior and Pacific slope regions, chiefly in its coastal character, its large representation of equatorial genera and in a relative absence of such northern genera as *Populus*, *Fagus*, *Corylus*, and many others which are so conspicuous in the so-called Fort Union flora and in other western floras of approximately the same age as the Fort Union.

Interest naturally centers in the probable origin of this exceedingly rich flora. As to this it may be said that from the known record the following genera had already attained a Holarctic distribution and were indigenous in southeastern North America at the beginning of Wilcox time:

<i>Acerates</i>	<i>Eugenia</i>	<i>Persea</i>
<i>Acorus</i>	<i>Euonymus</i>	<i>Pistia</i>
<i>Amygdalus</i>	<i>Euphorbiophyllum</i>	<i>Platanus</i>
<i>Anemia</i>	<i>Ficus</i>	<i>Poacites</i>
<i>Apocynophyllum</i>	<i>Fraxinus</i>	<i>Potamogeton</i>
<i>Aralia</i>	<i>Grewiopsis</i>	<i>Proteoides</i>
<i>Asplenium</i>	<i>Ilex</i>	<i>Prunus</i>
<i>Bunelia</i>	<i>Magnolia</i>	<i>Pteris</i>
<i>Celastrus</i>	<i>Marchantites</i>	<i>Rhamnus</i>
<i>Cinnamomum</i>	<i>Menispermites</i>	<i>Sapindus</i>
<i>Cissites</i>	<i>Myrcia</i>	<i>Smilax</i>
<i>Crotonophyllum</i>	<i>Myrica</i>	<i>Sparganium</i>
<i>Cyperacites</i>	<i>Nectandra</i>	<i>Sterculia</i>
<i>Dalbergia</i>	<i>Nelumbo</i>	<i>Taxites</i>
<i>Diospyros</i>	<i>Nyssa</i>	<i>Ternstroemites</i>
<i>Dryophyllum</i>	<i>Oreodaphne</i>	<i>Zizyphus</i>
<i>Dryopteris</i>	<i>Oreopanax</i>	
<i>Equisetum</i>	<i>Paliurus</i>	

Although the foregoing genera were indigenous, many of their species, especially in the more prolifically represented genera, were new products of evolution or were immigrants into this region.

For example, in the large genus *Ficus*, only three or four species are considered to have been indigenous, eleven are either new or are invaders from equatorial America, and four were derived from the western United States.

About 60 per cent of the genera, with over 100 species, are considered to have entered the region from equatorial America, either by way of Mexico, the Antilles, or as drift seeds and fruits.

The climatic conditions on the east and west coasts of the Mississippi embayment resulted in considerable differences in the floras along the two shores. Thirty-three genera with 37 species are confined to the western shore, and 148 genera with 354 species are confined to the eastern shore. The plants of the former show a greater resemblance to members of the contemporaneous floras of western North America and to present day floras of Central America; those of the latter are more closely allied to present day floras of northern South America, and presumably entered the region, at least in part, by way of the extended Antilles.

The Wilcox was a time of fluctuating but very shallow seas, even in southern Alabama where the most complete marine section is displayed. In the upper reaches of the embayment there were extensive sand flats, barrier beaches, coastal lagoons and estuaries, interspersed with swamps. It was in the last of these that plant debris accumulated to form the lignites which are so common in the Wilcox from Alabama to Texas, and which in places are sufficiently thick and pure to form the basis of coal mining.

The shallowness of the embayment waters at this time and the vast quantities of fresh water brought in by the master streams, draining practically the whole interior of North America south of the Canadian shield, prevented extension of the marine faunas of the Wilcox beyond the lower part of the embayment.

The Wilcox epoch is brought to a close by a withdrawal of the shallow Wilcox sea, and after a considerable interval, during which the coast line was an unknown distance south of the region, a second northward transgression of marine waters brought about a renewal of sedimentation over a considerable part of the Wilcox area. Sediments of this sea contain representatives of a middle Eocene marine fauna and samples of the contemporaneous terrestrial flora which clothed its shores.

THE MIDDLE EOCENE

The middle Eocene formations comprise what is known as the Claiborne group and their equivalents. Aside from petrified wood, the plants from the middle Eocene are much more limited in number and are present (preserved) at fewer outcrops than is the case in the lower Eocene. Claiborne plants are known from only 23 localities, scattered from the Chattahoochee River in Georgia to the Rio Grande region in Texas and northern Mexico.

The total number of known species is only 90 in 66 genera, 34 families, and 24 orders. These comprise a fungus, 6 ferns, 4 gymnosperms, 8 monocotyledons, and 71 dicotyledons. The largest families in the order of their relative importance are the Lauraceae, Leguminosae, Sapindaceae, Arecaceae, Polypodiaceae, Moraceae, Rhamnaceae, Combretaceae, Rutaceae and Celastraceae.

Some idea of the botanical character of the Claiborne flora can be given by an enumeration of some of the genera represented. These are

<i>Acrostichum</i>	<i>Fagara</i>	<i>Nectandra</i>
<i>Anemia</i>	<i>Ficus</i>	<i>Nyssa</i>
<i>Bactrites</i>	<i>Geonomites</i>	<i>Oreodaphne</i>
<i>Carapa</i>	<i>Glyptostrobus</i>	<i>Oreopanax</i>
<i>Cedrela</i>	<i>Goniopteris</i>	<i>Persea</i>
<i>Citrophylum</i>	<i>Inga</i>	<i>Pisonia</i>
<i>Coccobolis</i>	<i>Laguncularia</i>	<i>Reynosia</i>
<i>Combretum</i>	<i>Lygodium</i>	<i>Sapindus</i>
<i>Conocarpus</i>	<i>Mespileodaphne</i>	<i>Sophora</i>
<i>Copaifera</i>	<i>Mimusops</i>	<i>Sterculia</i>
<i>Diospyros</i>	<i>Momisia</i>	<i>Terminalia</i>
<i>Dodonaea</i>	<i>Myrcia</i>	<i>Thrinax</i>
<i>Eoachras</i>	<i>Myrica</i>	<i>Zizyphus</i>

The climate appears to have become progressively warmer during Claiborne time, and less than a dozen species of the extensive lower Eocene flora of this region have been detected in the middle Eocene. This reflects, in part, the limited extent of the flora known from the middle Eocene, and emphasizes somewhat the lapse of time between the lower and the middle Eocene times of sedimentation.

Since this middle Eocene flora is so distinctly a warm coastal flora it offers but slight resemblance to the Eocene floras known from other parts of North America, those which show some community being found in the Green River and Bridger basins of

Colorado and Wyoming; they are of approximately the same age as the Claiborne.

The Claiborne epoch is marked toward its close by a shallowing and ultimately by a considerable withdrawal of the Claiborne sea during which there were widespread accumulations of lignitic deposits in palustrine environments. The time involved in this interval, however, was much shorter than that between the lower and the middle Eocene.

THE UPPER EOCENE³

The sediments of upper Eocene age in the Mississippi embayment constitute the Jackson group of formations. They are inaugurated by a marked transgression northward of marine waters up the Mississippi valley, whose sediments overlap those of the Claiborne sea and extend as far as western Kentucky and northeastern Arkansas.

Recognizable plants of this time have been found from near the Savannah River in Georgia (Grovetown) southwesterly to Webb County, Texas. The number of named species is 133 which is somewhat more than is known from the middle Eocene, but very much fewer than from the lower Eocene, and altogether too few for purposes of either precise correlation or ecological deduction. These comprise 4 fungi, a specimen of *Marchantites*, 4 ferns, an *Equisetum*, 2 or 3 gymnosperms, 15 monocotyledons, and 106 dicotyledons. They represent 89 genera in 52 families and 32 orders. The larger families in the order of their importance are:

Lauraceae	Sapotaceae	Juglandaceae
Leguminosae	Rutaceae	Nyctaginaceae
Arecaceae	Sapindaceae	Combretaceae
Moraceae	Rhamnaceae	Myrtaceae

Thirty-seven of these Jackson species were already present in Claiborne time and continued into the upper Eocene.

The climate in this region is believed to have reached its maximum of geniality during the upper Eocene, or possibly in the succeeding Oligocene epoch, the known flora of the latter being too scanty to permit a decision on this point. Jackson floristics indicate three principal kinds of plant association. These are represented by estuary accumulations with lignites, often of considerable thickness and marking the sites of *Acrostichum* swamps, this genus being

³ Berry, E. W. U. S. Geol. Survey, Prof. Paper 92. 1924.

especially abundant; or by Mangrove swamps with their border plants (*Rhizophora*, *Conocarpus*, *Combretum*, etc.); or by beach jungle associations (*Thrinax*, *Sapindus*, *Dodonaea*, *Pisonia*, *Sapindus*, *Terminalia*, *Fagara*, *Lygodium*, *Cedrela*, etc.); all along with other plant types of no certain provenance. Petrified palm wood is especially abundant in the Jackson and is perhaps only a correlative of the prevailingly sandy nature of the deposits in the non-marine part of the area of outcrop, e.g., in the Texas region.

Plant genera present which subsequently became extinct in North America include the date-palm (*Phoenicites*), represented by broken rays and characteristic seeds in hard fruits; *Engelhardtia*, that curious genus of the Juglandaceae with winged fruits, restricted in modern floras to a single species in Central America which is sometimes made the type of a distinct genus, and to a dozen or more oriental species in the southeastern Asiatic region; characteristic fruits and seeds of nutmeg (*Myristica*); fruits of the Nipa-palm (*Nipadites*), now monotypic on tidal shores from India to the East Indies, but almost world-wide in the early Tertiary; twigs of what has been identified as *Glyptostrobus*, now monotypic in eastern China; and other and less spectacular genera.

During upper Eocene, and possibly extending into Oligocene time, there occurred the greatest northward extension of floras from equatorial America. In common with the earlier Tertiary floras of southeastern North America they appear to be more closely related to the existing flora of northern South America than to those of Central America or the Antilles, and it is believed that subsequent restriction of land areas in the Antilles is one of the reasons for this. Unfortunately, paleobotanical knowledge of all of equatorial America before the later Tertiary is practically non-existent.

This northern extension of equatorial forests alluded to in the preceding paragraph appears to correspond in time to the great polar extension of temperate floras into the Arctic region and possibly into the Antarctic as well (Seymour Island), happenings, the discovery of which awakened an interest that has continued unimpaired through several generations and is still the subject of frequent discussion and a still greater amount of misunderstanding.

It is believed by many, including the present writer, that, following the world-wide submergence of continental areas and the opening of free seaways between equatorial and Arctic oceans during

the middle Eocene, the North polar ice cap (the Antarctic, being a region of land and not ocean as is the Arctic, can not safely be included in this statement) was greatly reduced or disappeared altogether. The subject is too complex to be more than referred to in the present abstract, but the reader is not to misunderstand what has been said. These polar floras do not indicate uniform or tropical climates and a lack of climatic zones, and the numerous statements of such conditions that are contained in the literature are false.

The following bit of evidence, confirmatory of the interpretation given in the present paper, is worth recounting. A considerable number of Miocene floras are known from Cuba, Hayti, Porto Rico, Trinidad, southern Mexico, Costa Rica, Panama, Venezuela, and Colombia, and only a single Eocene flora in the whole region. This last is in Venezuela and, though extremely limited, it contains several types which are identical, even as to species, with those of the upper Eocene of the Mississippi embayment, whereas the widespread and much more extensive Miocene floras from equatorial America are wholly unlike those of the United States. One is, I think, justified in the belief that the interpretation is sound and that it would be confirmed if more were known of the older Tertiary paleobotanical history of equatorial America.

Returning more closely to the theme of the present paper, it may be noted that Eocene fossil plants northeast of the embayment region comprise an occasional fruit or seed in the marine deposits of Maryland and Virginia, and those from the isolated and unique lignitic basin at Brandon, Vermont. The Aquia and Nanjemoy formations, highly fossiliferous marine deposits in tidewater Maryland and Virginia, contain occasional drift fruits brought in by rivers from the adjacent mainland. The most abundant of these, resembling some of the forms from Brandon, Vermont, goes by the botanically nondescript name of *Carpolithus marylandicus*, and its botanical affinities remain to be discovered. Recently, a pine cone and a beautifully preserved woody fig fruit have been described from these deposits.⁴

The Brandon, Vermont, plants constitute a curious assemblage of fruits and seeds—long known and discussed in the earlier days

⁴ Berry, E. W. Jour. Wash. Acad. Sci. 24: 182-183. 1934; 26: 108-111. 1936.

of American paleobotany by Hitchcock (1853), Lesquereux (1861), Knowlton (1902) and others. Attempts to utilize these lignites for fuel during a coal shortage a generation ago led to the discovery of a very large amount of new material which was described by the late George H. Perkins, who for so long was State Geologist of Vermont. Although somewhat over-elaborated specifically, his work affords, nevertheless, a most interesting, and in fact the only, glimpse of the flora of New England during any part of the Tertiary.⁵

The Brandon flora was long thought to be of Miocene age, this age, for reasons that need not be enumerated here, having also been assigned to the Arctic fossil floras and to those of the western interior of North America, and even to those of the Mississippi embayment, all of which have subsequently been shown to be Eocene in age. The general climatic considerations that have been enumerated, the dissimilarity of the Brandon plants to known Miocene floras and climates, and the similarities and in several cases the identities between Brandon plants and those of the Wilcox, clearly show it to be Eocene.

Although the identical species are chiefly those of the Wilcox, this is regarded as due to the fact that the Wilcox flora is so much more extensive than those that are known from the Claiborne and Jackson, and was probably the source from which many of the Brandon plants spread to Vermont during the northward spread recorded in the middle and upper Eocene. My own opinion is that the Brandon deposit is of upper Eocene age;⁶ a comparison of the Brandon plants with the recent flora of Vermont or with the flora known from the Miocene of Maryland and Virginia, altogether precludes a Miocene age.

About 175 so-called species from Brandon have been described. The bulk of these are based upon fruits and seeds, although 2 or 3 woods have been described. Leaves have been found but these are poorly preserved and none capable of identification has been discovered. As will be seen from the appended list of genera many are form-genera whose relationship to recent genera has remained entirely problematical, although various students from Lesquereux's time onward have searched through collections of recent carpological material.

⁵ Perkins, G. H. Rep. State Geol. (Vermont) for 1903-1904; 1905-1906.

⁶ Berry, E. W. Am. Jour. Sci. 47: 211-216. 1919.

The following genera have been enumerated and the names will give sufficient indication as to which are form-genera of unknown affinity, which are supposedly related to living genera, and which belong to living genera:

<i>Apeibopsis</i>	<i>Drupa</i>	<i>Pinus</i>
<i>Aristolochia</i>	<i>Hicoria</i>	<i>Pityoxylon</i>
<i>Aristolochites</i>	<i>Hicoroides</i>	<i>Prunoïdes</i>
<i>Bicarpellites</i>	<i>Illicium</i>	<i>Rubioides</i>
<i>Brandomia</i>	<i>Juglans</i>	<i>Sapindoides</i>
<i>Carpites</i>	<i>Laurinoxylon</i>	<i>Sclerotites</i>
<i>Glossocarpellites</i>	<i>Lescuria</i>	<i>Staphidoïdes</i>
<i>Cinnamomum</i>	<i>Monocarpellites</i>	<i>Tricarpellites</i>
<i>Cucumites</i>	<i>Nyssa</i>	

THE OLIGOCENE

In eastern North America recognizable Oligocene sediments are confined to the southern states. Southwest of eastern Louisiana these sediments are almost entirely continental in character and thus far have yielded no fossil plants except petrified wood.

In Mississippi the Jackson epoch is terminated by a littoral sandy formation known as the Forest Hill sand which contains a few fossil plants intermediate in character between the upper Eocene and the Oligocene, and it is believed that this sand was partly contemporaneous with the uppermost Jackson and the lower Vicksburg marine sediments.⁷

East of the Mississippi River the Vicksburg, which is the group name for the Oligocene formations of the region, consists almost wholly of marine sediments, but that there was considerable oscillation of the strand at the close of the Eocene is indicated by the lignites which are often present at the base of the Vicksburg.

Since so much of the Oligocene is marine, and fossil plants are rare or have remained undiscovered in the western outcrops of continental materials, the known flora is exceedingly meager. About a dozen Jackson species are known to continue into the Oligocene, and petrified palm wood is especially abundant. The general facies of the Oligocene flora,⁸ if the few known species can be relied on for such a generalization, is much the same as that of Jackson time, i.e., a subtropical strand flora. Prominent members of the known flora are species of

⁷ Berry, E. W. U. S. Geol. Survey, Prof. Paper 92: 96. 1924.

⁸ Berry, E. W. U. S. Geol. Survey, Prof. Paper 98: 227-251. 1916.

<i>Acrostichum</i>	<i>Mimosites</i>	<i>Palmoxylon</i>
<i>Apocynophyllum</i>	<i>Myrcia</i>	<i>Pisonia</i>
<i>Fagara</i>	<i>Oreopanax</i>	<i>Sabalites</i>
<i>Ficus</i>	<i>Paliturus</i>	
<i>Lygodium</i>	<i>Palmocarpion</i>	

THE MIocene

Marine deposits of Miocene age, often with extensive marine faunas, are found from New Jersey southward, but, except for drift-wood or an occasional water-logged fruit or seed, they rarely contain identifiable traces of the vegetation which clothed the land. Consequently, our present knowledge of the Miocene floras of eastern North America is deplorably unsatisfactory when compared with what is known of the flora of this age in western North America or in Europe.

At Alum Bluff on the Apalachicola River in central Florida and near Hattiesburg in southern Mississippi we get a glimpse of the flora that flourished in those regions at the beginning of Miocene time.⁹ The first of these is especially interesting, even though the variety of plants is limited, since at this locality there is preserved a fragment of the coast just as it was emerging from the sea. The littoral sands are packed in places with the frayed and tangled rays and stipes of a fan-palm, and the shores were evidently covered with palmetto swamps or brakes. Other plants represented are beach plants, and others apparently came from the shores of nearby bayous. As far as it is known this flora would find a congenial habitat at the present time in the delta of the Apalachicola River or almost anywhere along the coast of peninsular Florida. This statement will give a fair idea of the predicated physiography and climate.

Beginning with the Cenozoic we have seen a long interval lasting through the Eocene and into the Oligocene during which the prevailing direction of plant dispersal was northward from equatorial America, which movement penetrated and largely replaced the temperate types which had inhabited the region during the Upper Cretaceous. At Alum Bluff and Hattiesburg we see for the first time the beginning of a reversal in the prevailing direction of this movement, for at these localities a few equatorial types linger, but these are apparently being replaced by temperate types coming in from the north.

⁹ Berry, E. W. U. S. Geol. Survey, Prof. Paper 98: 41-59.

Although the evidence is confessedly incomplete, it seems clear that this reverse movement had probably been going on for a short time, and that it continued on a large scale for a considerable time. This is indicated by the presence at the base of the Alum Bluff section of a warm-water marine fauna in a formation known as the Chipola marl and by a marine submergence following the emergence chronicled in the plant bed. This submergence is chronicled by a formation toward the top of the bluffs known as the Choctawhatchee marl, containing a marine fauna like that found in the Chesapeake group of Maryland and Virginia, which fauna is a distinctly cooler-water fauna than that of the Chipola. This conclusion is indicated also by the fact that the only Pliocene flora that is known from this region—that known as the Citronelle in western Florida and southern Alabama—is wholly modern in character.

The Alum Bluff-Hattiesburg early Miocene flora is not extensive, the genera represented being:

<i>Artocarpus</i>	<i>Fagara</i>	<i>Sabalites</i>
<i>Bumelia</i>	<i>Nectandra</i>	<i>Sapotacites</i>
<i>Caesalpinia</i>	<i>Pestalozzites</i>	<i>Ulmus</i>
<i>Cinnamomum</i>	<i>Pisonia</i>	
<i>Diospyros</i>	<i>Rhamnus</i>	

The only additional Miocene plants known from eastern North America, except marine diatoms whose frustules make up heavy beds of certain localities, are those which occur sparingly in the near shore deposits of the marine Chesapeake group which are of early middle Miocene age. Determinable leaves have been found at but two localities, one in the suburbs of Richmond, Virginia, and the other in the District of Columbia just southeast of Washington. Both are in the Calvert, the oldest formation of the Chesapeake group.¹⁰

The plants found at Richmond clearly indicate that the coast was low and was lined at this point with estuary cypress swamps, *Taxodium* being the most abundant type represented. Other genera more sparingly preserved at this locality are

<i>Carpinus</i>	<i>Nyssa</i>	<i>Rhus</i>
<i>Celastrus</i>	<i>Planera</i>	<i>Salix</i>
<i>Ficus</i>	<i>Platanus</i>	<i>Salvinia</i>
<i>Fraxinus</i>	<i>Quercus</i>	<i>Ulmus</i>

¹⁰ Berry, E. W. U. S. Geol. Survey, Prof. Paper 61-73.

Those found in the District of Columbia appear to be, for the most part, plants of coastal dunes, mixed with river-borne drift, and include *Berchemia*, *Cassia*, *Ilex*, several small-leaved oaks, cypress twigs, seeds of a pine, *Pieris*, *Rhus*, and various leguminous leaflets. Elsewhere the Calvert formation has furnished an occasional pine cone, cherry stone, acorn or walnut. Generically, all of these are very modern but several of the species are very similar to or identical with species from the middle Miocene of other regions. Climatically, these middle Miocene plants from Maryland and Virginia would be at home at the present time at almost any suitable locality south of the Potomac River, and I have visited many localities from the coast of North Carolina to Alabama that support a rather similar plant assemblage.

A considerable flora is known, but has never been described adequately, from beds in southern New Jersey that are known locally as the Bridgeton sandstone, from the town of that name near which they were found.¹¹ These have been variously considered to be late Miocene or Pliocene in age. Their similarity to the plants found in the Pleistocene Pensauken formation of New Jersey appears to indicate that they also are Pliocene in age, and hence outside the scope of the present paper.

THE PLIOCENE

Highly fossiliferous marine formations of Pliocene age are found in the Carolinas, in Florida, and at a few other localities near the present coast, but no land plants have been discovered in them. As was the case during the Miocene, little is known of the Pliocene floras of eastern North America as compared with our knowledge of floras of this age in western North America or Europe.

The only considerable Pliocene flora known from the whole Atlantic region, and that not an extensive one, has been found in the clays of what is known as the Citronelle formation in western Florida and southern Alabama.¹²

Eighteen species have been determined from these outcrops. The genera represented are:

¹¹ Hollick, A. Bull. Torrey Bot. Club 19: 330-333. 1892; 23: 46-49. 1896; 24: 229-231. 1897.

¹² Berry, E. W. U. S. Geol. Survey, Prof. Paper 98: 193-208. 1916.

<i>Betula</i>	<i>Hicoria</i>	<i>Quercus</i>
<i>Bumelia</i>	<i>Nyssa</i>	<i>Taxodium</i>
<i>Caesalpinia</i>	<i>Pinus</i>	<i>Trapa</i>
<i>Fagus</i>	<i>Planera</i>	<i>Vitis</i>
<i>Fraxinus</i>	<i>Prunus</i>	<i>Yucca</i>

The bald-cypress, water-oak and water-elm still exist in the region but the remainder are represented by extinct species. These are practically all similar to still-existing species of the mesophytic forest region of the southeastern United States except *Trapa* which no longer is a native of North America.

This Pliocene flora is comparable in a broad way with the existing flora of the same region. The forms thus far known are such as are found in modern times in cypress ponds, around coastal lagoons and with a sprinkling of forms found in live-oak thickets. It is concluded that the climate could not have been appreciably different from that which prevails in southern Alabama at the present time.

THE BOTANICAL REVIEW

VOL. III

FEBRUARY, 1937

No. 2

THE MIGRATION OF SOLUTES

T. G. MASON and E. PHILLIS

Cotton Research Station, Trinidad, B. W. I.

INTRODUCTION

By the middle of the nineteenth century the nature and sources of the raw materials used in the growth of green plants began to be appreciated. To-day it is established that the bulk of the carbohydrate is manufactured in and exported from the leaf, and that all the mineral elements are absorbed by and exported from the root. It is also recognized (*cf.* 7, 11) that the bulk of the mineral elements finds its way to the leaves from which some may be re-exported either in organic combination (*e.g.*, nitrogen, phosphorus, sulphur) or unchanged (*e.g.*, potassium, chlorine) to other parts of the plant. It is not denied, of course, that much of the nitrogen, etc., may be transformed into organic compounds before export from the root. Thus, the leaf may be regarded as the great distributing centre for food materials. It will be convenient, therefore, to consider the subject of solute migration under the following heads: (A) The export of mineral elements from the root; (B) The export of carbohydrate and mineral elements from the leaf; (C) The interchange of solutes between tissues. No attempt will be made to discuss the movement of hormones and related substances.

A

THE EXPORT OF MINERAL ELEMENTS FROM THE ROOT

Since the time of Hales, 1727 (5), the view has prevailed that the mineral elements absorbed by the root are transported upwards in the wood with the transpiration current. Further, as the bulk of the transpiration current travels to the leaf, for the transpiration losses from the leaf are much greater than from other organs, it seemed reasonable to assume that the bulk of the mineral elements would also be carried to the leaf. It is the peculiar service of Curtis

(2) that he has emphasized the meagre nature of the evidence on which this view is based. His experiments have, on the contrary, led him to suggest that soil solutes ascend the stem in the phloem. It is remarkable that about two hundred years after Hales' demonstration that the transpiration current ascended in the wood, there was no actual demonstration that soil solutes ascend also in this channel. Curtis has pointed out that the transport of dyes in the wood after their introduction through cuts and wounds hardly affects the issue. Moreover, the presence of inorganic solutes in the sap centrifuged or displaced from tracheae might be due to leakage from adjacent living cells. It must be emphasized, however, that Curtis has never succeeded in demonstrating that *soil solutes* can ascend the stem in the phloem. His experiments (3) indicating upward movement of solutes through defoliated stems, in which continuity of the wood was interrupted, demonstrate only that *storage materials* may travel upwards in the phloem from the region below the cut. Curtis relies, however, largely on experiments in which a ring of bark is removed so that only the wood remains to convey solutes. In his numerous ringing experiments he has always found that the leaves above the ring contain less nitrogen and ash materials than control plants. In the one experiment in which the stem was sampled it, too, was found to contain less nitrogen than controls. This he thinks indicates upward movement of solutes in the phloem.

Maskell and Mason (7, 9) obtained quite different results in their ringing experiments, for they found that the leaves and stem above the ring contain more nitrogen than in unringed controls. A number of explanations (*cf.* 6, 12) have been advanced to explain the difference in the results obtained by Curtis and by Maskell and Mason. Curtis (4) himself suggests that as the whole foliage region was separated from the root by a ring in the experiments of Maskell and Mason there must have been carbohydrate starvation below the ring, and that this would lead to the release of nitrogen into the tracheae. Had this occurred, there might of course have been an accumulation of nitrogen in the leaves and stem of the ringed plants in excess of that in the control plants, though it scarcely seems probable that this release of nitrogen could have exceeded the normal supply which he thinks ascended in the phloem. Moreover, he has overlooked the fact that a degree of carbohydrate

starvation sufficiently acute to lead to the release of solutes into the transpiration current should also lead to a diminution in the rate of solute uptake from the soil and that this should offset any release of solutes into the vessels. It should here be explained that Curtis in his experiments guarded against carbohydrate starvation of the roots by ringing only branches.

As a result of recent experiments in which the whole plant, both above and below the ring, was analyzed, we are inclined to attribute the disagreement between the results of Curtis and of Maskell and Mason mainly to the fact that the former did not sample his plants for a matter of weeks or months after the operation of ringing, while the latter sampled their plants after a period of days or weeks. In the Curtis type of experiment, in which only a branch is ringed, the ringed branch has to compete for water and salts dissolved in it, with unringed branches. Ringing, as Curtis himself points out, leads to a reduction in transpiration. The ringed branch thus obtains progressively not only less water but less salts than unringed branches. In the Maskell and Mason type of experiment, in which the whole foliage region is separated from the root by a ring, there is first of all an accumulation of salts in the region above the ring in excess of that in unringed plants. After a matter of a week or less there is, however, a diminished uptake of salts from the soil as a result of carbohydrate starvation of the root. When this occurs the salt content of the ringed plants declines below that of the normal plants. It will be clear that this starvation factor might, if carbohydrate transport is much localized, lead to a diminished uptake of salt by the roots supplying the ringed branches used by Curtis.

The first worker actually to demonstrate that soil solutes do ascend in the wood apparently was Clements (1). He ringed the branches of grapes and plum and the canes of three varieties of raspberries early in spring before the new shoots had formed. He found that the nitrogen content of ringed branches had increased at the end of the season by as much as 220 times the initial value, while the ash content during the same period had increased up to 90.5 times. It will be observed that while Curtis and Maskell and Mason compared ringed and unringed plants, or rather parts of plants, Clements compared branches before and after ringing and analyzed the whole region above the ring. Thus, Clements has

demonstrated that solutes do ascend in the wood, but he has not shown that they may not also ascend in the phloem, for he had no controls with which to compare his ringed branches. Further, it is not quite certain, though it is very probable, that the solutes which crossed the region of the ring in his branches came directly from the soil, for they have been released into the transpiration current from the stem below the ring. That solutes after absorption by the root ascend the stem in the wood is rendered very probable, however, by an experiment reported by Mason, Maskell and Phillis (10). Using defoliated stems with a ring separating stem and root and growing in a saturated atmosphere, they found that the stems and roots of ringed and control plants increased their nitrogen contents at approximately the same rate. They also found that when the wood instead of a ring of bark was removed little if any nitrogen ascended the stem.

To sum up, ringing experiments have shown that soil solutes ascend the stem in the wood, but they have not demonstrated that they may not also ascend in the phloem. It must be admitted, however, that the evidence available renders it very unlikely that they normally do so. Now it is not immediately apparent why they should not ascend the stem in the phloem, for it is beyond dispute that movement in the phloem is not polarized and that storage nitrogen like other solutes may travel either up or down the stem in this channel. There would appear to be two possibilities. First, soil solutes may enter one tissue predominantly as a result of differences in membrane permeability between wood and phloem (*cf.* 6). This explanation does not accord with the fact that potassium and chlorine appear to be transported upwards in the wood and then exported from the leaf in the phloem (8, 9). Secondly, it is possible that soil solutes may enter both wood and phloem and that upward transport of solutes in the wood to the leaf may stop upward transport in the phloem by destroying the net gradient between root and leaf. Upward transport in the wood is normally very rapid, and on arrival at the bundle ends solutes may be concentrated directly by the enlarged companion cells which are found there. This might explain why soil solutes such as potassium and chlorine, which do not appear to be chemically transformed, and why nitrogen, if transformed to amino acids in the root, do not ascend the stem in the phloem, but would not account for the apparent diffi-

culty of nitrogen to move upwards in the phloem from the root when the wood is broken, for in this case there should be a greater concentration of nitrogen in the phloem of the root than in that of the stem. It will be obvious that much further data is required before these problems can be solved.

B

THE EXPORT OF CARBOHYDRATE AND OF MINERAL ELEMENTS FROM THE LEAF

It is just a hundred years since Hartig's (18) discovery of the sieve-tube. His further discovery (19, 20) that sap exudes from the sieve-tube on cutting the phloem laid the foundation of the hypothesis of a *mass flow* through the sieve-tube. Sachs (57, 58), as a result of microchemical observations on the distribution of starch and protein, concluded that the former travelled in the starch sheath and the latter in the sieve-tube. Thus originated the hypothesis of *dual channels*. It is noteworthy that a mass flow of protein through the sieve-tube has until recently been widely accepted, no doubt because of the high concentration of protein in the sieve-tube, its non-diffusible nature and the belief that the sieve-pores are open. On the other hand, there has been great divergence of opinion as to where and how carbohydrates travel. Thus Sachs appears to have thought that sugars diffuse from one starch grain to another in the starch sheath. De Vries (12) recognized that diffusion was too slow to account for transport of carbohydrates and suggested that movement might be accelerated through the parenchyma by protoplasmic streaming. Strasburger (65) concluded that the sieve-pores of angiosperms were open but that the much smaller pores of conifers were closed. It is not clear how the advocates of a mass stream of protein through the sieve-pores thought that nitrogen travelled in conifers. Strasburger also concluded that protoplasmic streaming ceased in the mature sieve-tube.

Czapek (10, 11) rejected the hypothesis of dual channels. He demonstrated experimentally the slowness with which sugar moved through parenchyma cells and concluded that both nitrogen and carbohydrate travelled through the sieve-tube. He did not question the mass stream of protein through the pores, but thought that carbohydrate (and soluble nitrogen (11)) travelled through the cytoplasm of the sieve-tube and the cytoplasmic lining of the open pores.

He noted that narcotics slowed up carbohydrate transport and that plasmolysis of the sieve-tube did not stop it. He was thus led to emphasize the importance of metabolism and concluded that sugars travel by a process akin to secretion. In rejecting protoplasmic streaming as a factor, he of course followed Strasburger. Mangham's (29) views were not unlike those of Czapek. He did not question the mass flow of protein through the sieve-pores, but thought that carbohydrate travelled through the cytoplasm. He considered that sugars were adsorbed on the surfaces of colloids and travelled at enhanced rates along their surfaces. His views were adversely criticized (26) at the time, but a rather similar mechanism has been recently suggested by Clements (3). The physicist of to-day no longer regards materials as anchored when adsorbed, but admits that movement may occur on surfaces (cf. 67). Mangham recognized that sugar might travel from one sieve-tube to another without traversing the plasma membrane and, consequently, the impermeability of the plasma membrane (cf. 56) would not prove an obstacle in the way of carbohydrate movement from one sieve-tube to another.

About this time doubt began to arise concerning the ability of the sieve-tube to transport food materials. Thus Schmidt (60) challenged the generally accepted view (cf. 65, 21) that the sieve-pores of angiosperms were hollow and that the vacuoles of neighboring sieve-tubes were in direct communication with one another. His conclusion that the pore contained a solid core of cytoplasm (cf. 28) of course rendered very doubtful the current view of a mass movement of protein through the pore from one sieve-tube to another. Next Birch-Hirschfeld (1), Dixon and Ball (14), and Dixon (13) emphasized the inadequacy of the phloem for conduction. Dixon and Ball calculated that a sugar solution would have to traverse the phloem at a rate of 50 cm. per hour to supply a growing potato tuber. Rates of this magnitude they thought to be impossible through the phloem. They accordingly suggested, since diffusion was too slow and protoplasmic streaming reported to be absent, that food materials left the leaf and descended the stem in the wood. It had long been recognized (cf. 16) that water might travel backwards in the wood, but the suggestion that food materials normally travel in this way was something new. They dismissed all the evidence based on ringing experiments on the grounds

that ringing results in plugging of the outer xylem, where they suggested the backward movement of food materials occurred. Mangham (30) criticized the summary dismissal by Dixon and Ball of the phloem as "the main channel for the removal of the organic materials formed in the foliage." He referred, in particular, to the work of Schneider-Orelli (61) who showed that when the xylem of the vein was destroyed by a leaf miner of the apple, starch did not accumulate in the distal part of the lamina, but that injury to the phloem resulted in an accumulation of starch proportional to the injury. He also referred to the work of Quanjer (55) who found that the phloem necrosis associated with leaf curl of potatoes interferes with transport of starch from the leaf. He might also have alluded to the discovery of Hanstein (17) that ringing plants with internal phloem failed to stop transport.

Mason (37) showed that ringing checks carbohydrate transport into the tuberous roots of cassava and explained this by postulating that carbohydrate transport normally occurs in the wood and that a hormone necessary for the development of the tuber travels downwards in the phloem. An essentially similar explanation of the results of ringing experiments was published by Kastens (25) in 1924. In 1926 Mason and Lewin (39) showed that to supply a developing yam with carbohydrate through the phloem would require a mass flow of solution at rates even higher than those calculated by Dixon and Ball. In the same year, Mason (38) discovered the presence in the yam of compact balls of parenchyma, the bast glomeruli, which interrupt the sieve-tubes at every node. He thought that a mass movement of solution through the phloem of the yam was, therefore, impracticable.

In 1926 Münch (49) published a preliminary account of his *Druckstromhypothese*, which later he has amplified and elaborated (50, 51). He rejected Dixon's hypothesis of transport in the wood, for he considered that his own and earlier bark *flap* and *strip* experiments proved that transport occurred in the bark. He described a *strip* experiment in which bark and wood were isolated from one another by prizing apart the two tissues over a distance of several centimeters. From the continued growth of the isolated bark, he concluded that transport occurs in this tissue. He did not realize that the question at issue was not whether the bark can transport food materials, but whether it can conduct them at rates that

calculation shows must obtain in the intact plant. He pointed out with some justification that Dixon's rejection of the phloem as the channel on the grounds of its unsuitability was largely due to the fact that he did not conceive any motive power potent enough to drive sap through the sieve-tubes. Münch's hypothesis of a *Druckstrom* was not original (*cf.* 52), but the application of the osmotic mechanism suggested by Pfeffer (54) to explain exudation from a cell to drive sap through a series of cells had not before been suggested. His hypothesis postulated a movement of sap through the sieve-tube system from a region of high turgor pressure in the leaf to regions of lower pressure in the stem, etc. The production of sugar in the leaf should, it was thought, attract water from the wood and so generate turgor pressures in excess of those obtaining in regions where sugars were removed in growth, etc. The removal of sugars as transport proceeded down the stem would liberate water into the wood. Münch claimed that water is exuded from the inside of the bark when bark and wood are prized apart. On the whole, he produced very little evidence in support of his hypothesis, which is, however, the only hypothesis that approaches a complete explanation of motive power and mechanism of transport. The hypothesis assumes not only water exudation from the cambium, but also a turgor pressure gradient from leaf to root sufficient to overcome the resistance of the intervening tracts. It also postulates that the rôle of the sieve-tubes is a purely passive one and finally that all food materials travel in the same direction. How far these assumptions are justified was not determined by Münch. He of course relied mainly on the fact that many plants do exude sap from the sieve-tubes when the phloem is punctured and considered this evidence of movement in the intact plant. He observed that many plants do not show this exudation, but thought that under more favorable conditions they might do so.

In 1928 there appeared two papers by Mason and Maskell (40, 41) on the transport of carbohydrates in the cotton plant. The earlier workers on transport had relied on changes in growth or starch content. Mason and Maskell introduced chemical methods and estimated the weights of carbohydrate, and the sugar concentrations in the sap, in leaf, bark, wood, etc. In this way they were able to examine the changes in carbohydrate at much shorter intervals than had hitherto been possible. In their first paper, they were

concerned primarily with the channel of transport and in their second with the factors that determine rate and direction of transport. They concluded that the channel was the phloem and not the wood for the following reasons:

(1) The diurnal changes in the sugar content of leaf were reproduced a few hours later in the bark but not in the wood.

(2) On ringing the stem, the carbohydrate content and sugar concentration of the sap in leaf, bark and wood above the ring increased after only a few hours over that in unringed plants. Below the ring the carbohydrate content and sugar concentration in the sap of bark and wood diminished below that of control plants. Such a quick response to ringing, they thought, excluded damage to the wood, as suggested by Dixon, being the reason for the interruption of transport. They also observed that a dye moved down the wood past the region of the ring and from this concluded that the mechanism responsible for the backward movement of dyes in the wood and for carbohydrate transport were different. They also noted that though ringing increased the concentration of sugar in the wood as a whole, it did not affect the concentration of sugar in the sap in the tracheae. By subdividing the bark tangentially into three fractions they showed that the response to ringing was due mainly to sucrose in the inner part of the bark where of course the phloem is located. They concluded from this that sucrose was the main form in which carbohydrate is transported through the stem and that the phloem was the actual channel of transport.

(3) While complete removal of the bark stopped transport, isolation of the bark from the wood, by inserting paper between the two over a short distance, allowed transport to proceed at approximately normal rates. This showed that contact between bark and wood is not necessary for transport.

(4) Transport took place into levered-up flaps of bark at approximately the normal rate. Movement was detected before the appearance of new wood elements on the inside of the bark flap. In previous flap experiments of this type (*cf.* 51) the possibility was not excluded that new wood was formed from the bark and that transport then proceeded in this new wood.

As to the mechanism of transport, they concluded that transport follows a *diffusion pattern* in that the rate of transport is correlated with the sugar gradient in the bark and the direction of transport

in the bark is always from a region of high to one of low concentration. Further, changes in the sugar concentration in the leaf were followed by changes in the concentration in the bark and vice versa. Moreover, transport into the fruit was four times as rapid by day as by night. It was found, however, that the actual rate of movement through the phloem was about 40,000 times as rapid as would be expected from physical diffusion in water and that the total sugar concentration in the leaf was much less than in the bark. They make no reference to the work of Münch, but apparently believed that the arguments advanced by Dixon for the potato and by Mason and Lewin for the yam precluded the possibility of a mass stream through the sieve-tube. Of outstanding importance in their work is the fact that they established for the first time beyond doubt that, in spite of the difficulty of finding a satisfactory mechanism to account for the actual rate of transport, carbohydrate transport does take place through the phloem. The data on which they base the analogy with physical diffusion do not, however, exclude the Druckstrom mechanism of Münch.

Curtis (8) in 1929 published an important paper reaffirming Czapek's conclusion as to the importance of living cells in transport. He showed that chilling the petioles of bean leaves to between about 1° C. and 4°-6° C. stopped or greatly retarded the removal of carbohydrate from the leaf blade, while the rate of translocation was not much influenced as the temperature was lowered from 25° C. to 6°-8° C. He also showed that enclosing the petioles in tubes containing nitrogen checked transport. He concluded, like Czapek, that movement was dependent on living cells, but, unlike Czapek, he thought that protoplasmic streaming was responsible for the acceleration of diffusion in the sieve-tube. His hypothesis possesses the merit that it would accord with a diffusion plan of transport. Repeated failures (*cf.* 22) to observe streaming in mature sieve-tubes militates against this view, though this is not an insuperable objection, for the operation of cutting the phloem might, as Curtis emphasizes, disturb the mature sieve-tube more than other cells. A more serious objection is the high rate that would be required to produce the observed acceleration of diffusion. Mason, Maskell and Phillis (44) calculated that a rate of 336 cm. per minute would be required through the sieve-pores of cotton to accomplish this and that to supply the necessary energy, a 25 per cent. solution

of sucrose in the sieve-tube sap would be completely exhausted in one day. Further evidence that transport does not depend on streaming is supplied by Mason and Phillis' (47) observation that a degree of oxygen starvation that completely checks transport is without effect on the rate of protoplasmic streaming in the phloem parenchyma adjacent to the sieve-tubes. It would seem that Curtis' suggestion must be rejected and that *transport occurs through stationary cytoplasm.*

Maskell and Mason (31, 32, 33, 34, 35), in a series of papers on nitrogen transport, showed that nitrogen behaves like carbohydrate in that (1) diurnal changes in the leaf are reproduced a little later in the bark, (2) ringing leads to accumulation in the stem above the ring and diminution below it, (3) direction of transport in the stem may be reversed experimentally and (4) partial ringing leads to an increase in rate across the remaining bridge of bark. They concluded that, in so far as the gross phenomena went, nitrogen, like carbohydrate transport, follows a diffusion pattern. They found, however, that carbohydrate moving down the stem travelled down a gradient of total sugars, while nitrogen, also moving down the stem, travelled against a gradient of organic crystalloid nitrogen. They observed that the gradients of organic crystalloid nitrogen were positive in the wood and in the leaves, *i.e.*, the concentration was greater in the young than in the old leaves, and suggested that in the bark the negative gradient in organic crystalloid nitrogen consisted of a positive gradient of mobile or dynamic nitrogen which was *masked* by a steeper negative gradient of storage or static nitrogen. In support of this suggestion, they showed that:

(1) When carbohydrate and nitrogen transport down the bark was brought to a stop by removal of the leaves and ringing the stem near the ground, the sugar gradient disappeared but the gradient in organic crystalloid nitrogen remained negative.

(2) When the directions of carbohydrate and nitrogen transport in the stem were reversed experimentally, the sugar gradient in the inner part of the bark was reversed while the gradient in organic crystalloid nitrogen was steepened. This steepening of the nitrogen gradient they interpreted as the reversal of an originally positive dynamic gradient superimposed on a relatively static negative gradient. It was found that these changes in gradient occurred in the inner part of the bark where the phloem of course is mainly located.

In support of their hypothesis of masking is the observation of Mason and Phillis (45) that when the fruits develop, they draw on the nitrogen stored in the bark and the direction of the nitrogen gradient changes from negative to positive. Maskell and Mason (43) also found that during vegetative development the storage of nitrogen in the bark takes place very largely as amide nitrogen and that this fraction is responsible for the observed negative gradient in crystalloid nitrogen. A positive gradient, on the other hand, was found for the residual nitrogen fraction which may, they suggest, represent the mobile compound. In support of this suggestion they show that amide nitrogen occurs mainly in the rays and residual nitrogen in the phloem. A very important conclusion drawn by Maskell and Mason (33) is that the mechanism accelerating diffusion in the sieve-tube works impartially on carbohydrate and nitrogen. If further work confirms this conclusion, it would follow that nitrogen and carbohydrate travel by the same mechanism and in the same channel.

That materials other than carbohydrate and nitrogen are exported from the foliage leaf via the phloem was shown by Mason and Maskell (42) in 1931. They found that phosphorus and potassium accumulate in the stem above a ring and diminish below it, but that the distribution of calcium is unaffected by ringing. They concluded that phosphorus and potassium are mobile in the phloem and that calcium is not. Maskell, Phillis and Mason (36) extended these observations and found that in addition to carbohydrate, nitrogen, phosphorus and potassium, sulphur, magnesium and chlorine are phloem mobile. On ringing, they found that potassium, magnesium and chlorine leak with great ease into the vessels above the ring. It is sometimes difficult, consequently, to detect the mobility of these elements by means of the conventional ringing experiment. Using levered-up flaps of bark, however, the movement of these elements down the bark was detected. The apparent immobility of calcium in the phloem is of interest in view of the absence of this element in the exudate from sieve-tubes of cucurbits (27, 47).

In a valuable paper published in 1930, Schumacher (62) showed that when the margin of the leaf of *Pelargonium zonale* is cut and dipped into a dilute solution of eosin, the sieve-pores in petiole and stem are rapidly closed by callus, while protoplasmic streaming in

the phloem parenchyma remains unaffected. As under these conditions the export of nitrogen and carbohydrate is checked, Schumacher concluded that the transport of both these materials occurs in the sieve-tube. *This was the first experimental demonstration that the sieve-tube is the actual channel of transport of both carbohydrate and nitrogen.* It is of course uncertain whether the eosin actually travelled in the sieve-tube or whether it travelled backwards through the wood and spread outwards to the phloem. In 1933 (63) he used the relatively non-toxic dye fluorescein, traces of which can be detected in virtue of its property of fluorescing in ultra-violet light. When blobs of gelatine containing a dilute solution of the dye were placed on the scraped dorsal surface of a leaf vein, the dye spread downwards through the sieve-tube system of the plant. He found it present in the cytoplasm of the sieve-tube without a trace being visible in the vacuole or in the neighboring parenchyma. While his conclusion that transport occurs in the cytoplasm is very probably correct, he does not seem to have wholly excluded the possibility that the dye travelled backwards from the leaf in the wood and was then accumulated by the companion cell and the sieve-tube. Further, even if the dye did travel longitudinally in the sieve-tube, it does not of necessity follow that its presence in the cytoplasm and not the vacuole denotes that it travelled in the cytoplasm (*cf.* 15). Schumacher confirmed Schmidt's observation that the sieve-pores are filled with cytoplasm. It is noteworthy that a large number of plants fail to show this reaction to fluorescein. In Trinidad we have failed to reproduce Schumacher's results, even with *Pelargonium zonale*, the plant mainly used by him. As one of us has been privileged to see the fluorescein experiment carried out by Schumacher at Bonn and to confirm his observations, our failure in Trinidad is at present inexplicable.

Van den Honert (66) in 1932 suggested that changes in surface tension brought about by solution of assimilates and their removal at some remote point may cause a mass movement of solution, since any substance causing a lowering of surface tension causes a recession of the surface layer from that point. The interface between vacuole and cytoplasm was suggested as the probable surface in which movement takes place. The vacuoles, however, of neighbouring sieve-tubes are not continuous through the sieve-plates, and this would restrict the movement to a single sieve-tube. More-

over, the area available for movement would be very small and the boundary between cytoplasm and vacuole in the mature sieve-tube appears to be very nebulous (*cf.* 6). It is also difficult to see how solutes could move independently (44, 46) of one another by such a mechanism.

In a series of papers (4, 5, 6, 7) Crafts has developed the novel hypothesis that Münch's Druckstrom proceeds through both the sieve-tube lumen and its wall. He made the interesting observation that the walls of the phloem may occupy a greater proportion of the cross sectional area than the sieve-tubes. Thus in the potato he found that the walls occupy 32.5 per cent., the sieve-tubes 22.9 per cent. and the sieve-pores only .5 per cent. of the total cross sectional area. He also observed that the walls may shrink as much as 50 per cent. on dehydration. From calculations made of the resistance offered by the sieve-plate (pores assumed to be devoid of cytoplasm) and the wall, respectively, he concluded that the resistance of the latter was small relative to that of the former. His estimates of the resistance across the sieve-plates led him to believe that the resistance was too great for the turgor pressure gradients to force solution through them at the required rate, while the resistance of the wall was small enough to permit of the whole Druckstrom. Steward and Priestley (64) have pointed out that he seriously underestimated the resistance of the walls, and Mason, Maskell and Phillis (44) that he overestimated the resistance of the pores. The latter conclude "that the dimensions of the sieve-pores, except perhaps in the fine leaf-veins of dicotyledons and in conifers, are not in conflict with the mass flow theory, *provided the pores are normally open.*" Steward and Priestley point out that the exudation from the sieve-tubes observed by Crafts is probably "not an indication of a normally occurring mass flow at all, either in the walls or in the sieve-tubes, but may be due rather to the turgor of surrounding tissues and the release of tissue tensions consequent on cutting."

Crafts has also made the important observation that exudation may occur from sieve-tubes with callused sieve-pores. That water and solutes may be pressed through the plasma membrane and through the wall is easy to demonstrate by pressing leaf tissue in the jaws of a vise. Before the cells are disrupted, water and solutes, especially potassium, are then expressed. Crafts' conclusion that

such an exudation from the walls represents normal flow through the walls is of course unwarranted. The exudation observed by James and Baker (24) from the region of the cambium is possibly due to the same causes. Crafts confirmed the observation of Schmidt and Schumacher concerning the absence of inter-vacuolar canals in the sieve-pores. He also found that the distinction between vacuole and cytoplasm in the mature sieve-tube is not at all pronounced. It is interesting to speculate if this can be due to the absence of calcium in the sieve-tube. Crafts failed to plasmolyze sieve-tubes. We have found that in order to ensure plasmolysis of the sieve-tube it is necessary to carry out the operation on the bark before it is cut and removed from the plant. Crafts (7) has found that the exudation may exceed the volume of the sieve-tubes in the stem supplying it and has concluded, therefore, that the exudation is not of local origin, as Steward and Priestley suggest. He does not, however, show that all the exudate was supplied by the sieve-tubes. In his most recent paper he suggests that the Druckstrom flows through the whole phloem.

We have now to review a number of papers dealing with the Münch hypothesis. Weevers and Westenberg (68) failed to repeat Münch's observations on the exudation of water from the inside of flaps of bark levered-up from the wood but in communication above with bark still in contact with wood. Curtis and Scofield (9) found that the movement of materials from storage organs to growing regions proceeded against a gradient of osmotic pressure, and, they presumed, against a turgor pressure gradient. It seems, however, from the work of Phllis and Mason (53) that the companion cells may accumulate sucrose against a gradient. Curtis and Scofield's observations on osmotic (or turgor) pressure gradients between whole organs would not, therefore, invalidate the Druckstrom, for though the turgor pressure gradient between the whole organs or tissues might be negative, yet the gradient in the sieve-tube system might be positive. Probably the most serious experimental objection to the Druckstrom is that it requires a uni-directional stream of all solutes and that this requirement clashes with the observation of Mason, Maskell and Phllis (44) and of Mason and Phllis (46) that carbohydrate and nitrogen may travel simultaneously in reverse directions through the phloem. Mason and Phllis (47) have also confirmed Curtis' observations on the neces-

sity of oxygen for transport. They reduced the oxygen supply over a limited region of bark and observed that when the degree of starvation was considerable, transport might be completely checked.

The position to-day appears to be that we have indisputable evidence that the high speed longitudinal export of materials from the foliage leaf takes place through the sieve-tube system. There is also evidence that carbohydrate transport along the phloem follows a diffusion pattern in that direction and change in rate are determined by the gradient. The actual rate, however, is many thousands of times greater than would be expected as a result of physical diffusion in aqueous solution. Energy relations involving carbohydrate metabolism would appear to be responsible for this acceleration or activation of diffusion. Protoplasmic streaming cannot be involved and, consequently, transport must proceed through stationary cytoplasm.

Theoretically there would appear to be two general methods by means of which metabolic energy might be expended in activating diffusion. Firstly, it is possible to conceive of the solute itself utilizing the energy. Its free energy might be increased resulting in what might be termed solute projection. If metabolic energy is utilized in this way, it might be expected (1) that only the movement of metabolites would be accelerated in the sieve-tube, and (2) that the extent of this acceleration would depend on how intimately they enter into the metabolism of the cell. The second method by means of which respiratory energy might be spent in hastening diffusion involves the existence of some unknown organization in the cytoplasm, which for its maintenance requires a continuous supply of oxygen, whereby the resistance offered to solute movement is diminished. Such an organization would exist as long as metabolism was normal and the consumption of energy would be independent of the amount of material transported.

As to the mobility of materials in the sieve-tubes, we quote from our paper, "Oxygen Supply and the Activation of Diffusion" (47). "We have found that sugars, nitrogen, phosphorus, potassium, magnesium and chlorine are readily mobile in the phloem of the cotton plant, while calcium apparently is not. In an analysis of the exudate from the sieve-tubes of cucurbit stems, we found the above materials to be present, with the exception of calcium. It would appear that all materials in solution in the sieve-tube are mobile.

The mobility of chlorine is of particular interest, for this element appears to be completely in solution and in an ionizable form in the cotton plant and is usually considered to play no essential part in metabolism. Fluorescein, according to Schumacher, may travel through the sieve-tube at rates comparable to those of other solutes. Allusion may also be made to the work of Bennett, of Holmes, and of Caldwell on the transmission of viruses through the phloem. It may, we think, be concluded that substances other than metabolites are freely mobile in the phloem. Moreover, the rate at which they move appears to be much greater than can be accounted for, on purely physical grounds."

As to the extent to which the diffusion of various solutes is accelerated, we may refer to the work of Maskell and Mason. They say "the acceleration in the rate for unit gradient would seem to be about the same order for nitrogen as for sugars." As sugar and nitrogen are both very intimately associated with metabolism, this result might perhaps be expected, whether respiratory energy is utilized by the solute or by the medium. The position is perhaps different for nitrogen and magnesium. We found that the transport of magnesium, in company with phosphorus and potassium, was checked to a smaller extent than that of nitrogen and carbohydrate, and suggested that this difference was due to the fact that a larger proportion of phosphorus, potassium and magnesium than of nitrogen and sugar entering the ovule was supplied by the transpiration current. An alternative explanation now presents itself, for this difference could arise if the acceleration of metabolites at any level of oxygen supply varied with their importance in the economy of the cell."

In the same paper (47), an experiment is described in which an account is given of the effect of doubling the length of stem covered with plasticine. The results of this experiment were summarized as follows: "It was also found, taking nitrogen as the indicator of transport, that doubling the length of stem covered with plasticine diminished the amount of material transported in approximately geometrical progression. It is pointed out that this would occur if the degree of oxygen starvation was the same throughout the length of the stem covered with plasticine and if the acceleration of diffusion was the same in the two halves of the Long group as in the Short one. It is suggested that the mechanism activating diffusion

consists in some special organization in the cytoplasm, maintained by metabolic energy, whereby the resistance to solute movement is so reduced that materials diffuse in the sieve-tube at rates comparable with those in a gas."

Mason, Maskell and Phillis (44) point out that no calculation of the energy needed to expedite diffusion seems possible. It may, however, be accepted that the energy consumption would be enormous, provided that laws of classical physics obtain in the sieve-tube. It would, in fact, seem doubtful whether the carbohydrate present could supply the necessary energy. Huber (23) has emphasized this aspect of the problem. From this point of view the hypothesis of *Activated Diffusion* is untenable. It is in this connection worth while recalling Sach's warning. He said (59) "we often meet with the view, especially in modern times, that vegetable physiology is virtually only applied physics and chemistry, as though the phenomena of life could be simply deduced from physical and chemical doctrines. This might perhaps be possible, if physics and chemistry had no further questions to solve in their own domains, but in fact both are still as far distant from this goal, as physiology is from hers." To-day, however, the physicist recognizes "activated diffusion" under certain conditions. Reference may, for instance, be made to the work of Volmer (67) and of Bosworth (2). The latter has recently published a paper on the mobility of potassium on tungsten, in which he says "In broadest outline the theory for this type of activated diffusion postulates that an adatom is normally at rest on the surface and when suitably activated is raised to a mobile state, in which state it may move freely (*i.e.*, without doing work) over the surface until deactivated." It would, however, be premature to conclude that adsorption and surface forces are necessarily involved in the sieve-tube (*cf.* 3), for both sucrose and chlorine are phloem mobile and apparently completely in solution in the plant (48). For the present, we can only suggest that in the sieve-tube the cytoplasm as a whole is activated by metabolic energy and that it behaves as a liquid with diffusion constants enormously greater than those in water.

C

THE INTERCHANGE OF SOLUTES BETWEEN TISSUES

In Mason and Maskell's (8, 9) work on carbohydrate transport they found that the concentration of *total sugars* in the leaf was less

than in the bark. They thought that carbohydrate travelled down a gradient of reducing sugars from the mesophyll to the phloem of the fine veins and that in the phloem reducing sugars were condensed to sucrose. Along the bark of the stem they found that movement always occurred from a region of high to one of low sugar, sucrose as well as hexose, concentration. Transport from the bark to the ovule, like from mesophyll to vein, proceeded against a gradient of *total sugars*, but the *sucrose* gradient was markedly positive. Thus in their work they thought that actual transport into, out of and along the sieve-tube system, took place down a gradient. In their work on nitrogen transport (3, 4, 5, 6, 7) they found that transport down the bark of the stem proceeded against a gradient of organic crystalloid nitrogen. They interpreted this as being due to a positive gradient of residual nitrogen in the sieve-tube which was masked by a steeper negative gradient consisting mainly of amide nitrogen in the rays. Thus again they thought that actual movement occurred from a region of high to one of low concentration.

Loomis (2) has pointed out that during the period of most rapid translocation the gradients of total sugar and of all sugar fractions were negative for each tissue between the leaf blade and the cob of the young ear. Curtis (1) says that he and Scofield have obtained considerable evidence that growing tissues receiving sugars from the storage tissues may have concentrations of both sucrose and reducing sugars distinctly in excess of the supplying storage tissues. Neither Loomis nor Curtis and Scofield appear to have attempted to distinguish between the concentrations in the phloem and in the supplying and receiving tissues, respectively.

Maskell and Mason (7) attempted to discover why fertilized ovules take up carbohydrate and nitrogen more rapidly than unfertilized ovules. They observed that the concentrations of sucrose and residual nitrogen were greater in the bark (supplying) than in the ovules (receiving) in both fertilized and unfertilized ovules. They assumed from this and, of course, certain other considerations, that sucrose and residual nitrogen were the forms in which carbohydrate and nitrogen entered the ovules. They found that fertilized ovules had a lower concentration of residual nitrogen than ovules that had not been fertilized and suggested that the increased rate of nitrogen uptake as a result of fertilization might, therefore,

be due in part to an increased rate of nitrogen utilization, for an increase in the rate of nitrogen utilization should diminish the concentration of the mobile form of nitrogen and thus steepen the gradient into the ovule. This led them to suggest that the increased rate of nitrogen uptake, consequent on fertilization, could be explained on a gradient basis. For carbohydrate transport into the ovule a gradient explanation of the effects of fertilization on uptake proved inadmissible as the sucrose concentrations were found to be identical in fertilized and unfertilized ovules. They suggested that fertilization might facilitate in some way the ease of entry of sucrose. Thus they assumed that the ovular (receiving) tissues themselves play a relatively passive rôle.

Phillis and Mason (10) re-examined the mechanism responsible for the movement of carbohydrate from the assimilating cells of the leaf into the phloem of the fine veins. They concluded that movement occurred in the form of sucrose and that the concentration in the phloem was much greater than in the mesophyll. They suggested that the greatly enlarged companion cells in the fine veins were able to accumulate sucrose against a gradient from the border parenchyma and secrete it into the sieve-tube. Thus they thought that the movement of carbohydrate out of the leaf was polarized. They stressed the analogy with the accumulation of salts against a gradient and pointed out that energy relations involving carbohydrate metabolism must be involved.

In a more recent paper (11) they have shown that prior to anthesis, materials enter the corolla of the flower of the cotton plant, and that after anthesis there is a rapid movement of the same materials back into the body of the plant. From an examination of the concentration gradients between the corolla and the bark they concluded that the change in the direction of transport can not be explained on a purely gradient basis. They observed that at the time materials began to be exported from the corolla, its color changed from yellow to red and that this change in color spread gradually from the apex to the base of the petal. They noted that the veins remained yellow and turgid long after the parenchyma between the veins had reddened and suggested that the reversal of the direction of transport was associated with some change in the parenchyma rather than in the phloem. They were thus led to suggest that the tissues receiving from and supplying the phloem might also play an active part in the interchange of solutes.

A clue to the type of mechanism that might be at work was furnished by an earlier experiment (10) in which they attempted to reverse the *direction* of sugar transport in the foliage leaf. Examination of the concentrations in the phloem and adjacent tissues (*e.g.*, cortex of petiole) of illuminated and darkened leaves showed that the sucrose concentrations in the phloem and the adjacent tissues tended to remain constant relative to one another whether the leaf was illuminated or darkened and independently of the concentration levels. This, they point out, is what would happen if sucrose was distributed among the various tissues in accordance with the law governing the distribution of a solute between immiscible solvents. They were thus led to make the suggestion that the various tissues of the leaf behave as if they have different solvent capacities for sucrose. They stress the analogy between the accumulation of solutes by a cell from an external aqueous medium, as investigated by Steward (12, 13), and from other cells, as occurs in the movement of sucrose between the phloem and the adjacent tissues, and suggest that the mechanism may be the same in the two cases. They advance the hypothesis that though movement through a single cell or a single tissue (*e.g.*, the phloem) may be conditioned by concentration gradients, yet in the movement of solutes between tissues the solvent capacities of the tissues must play a part. They further postulate that not only the activation of diffusion through a tissue, but also the movement of solutes from one tissue to another, is dependent on some special organization of the cytoplasm which for its existence and maintenance requires the expenditure of metabolic energy. Thus they conceive of cytoplasm as a liquid possessing, in virtue of some unique organization, different diffusion constants and different solubilities to those of water.

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RECENT ADVANCES IN THE STANDARDIZATION AND IMPROVEMENT OF BIOLOGICAL STAINS

H. J. CONN

Chairman, Commission on Standardization of Biological Stains

HISTORICAL

The problem of standardizing biological stains did not occur to biologists previous to the War. Until German dyes were excluded from other countries it was quite generally accepted throughout the biological world that for staining purposes the dyes put out by Dr. Grübler of Leipzig were sufficiently standardized, although it has since been learned that these stains were not only lacking in uniformity but sometimes even mislabelled.

The need of standardization was first brought to the attention of American biologists by the exclusion of German dyes during the War and the post-War period of complete embargo on such products, when it was found that the domestic stains were not sufficiently uniform to be reliable. This problem did not become acute in America until the pre-War stock of German stains had become exhausted, which did not occur until about 1918-20. When German stains became available again in the United States it was found that another factor of uncertainty had been introduced, due to the fact that Dr. Grübler had fathered two concerns, one originally a laboratory manufacturing certain biological products, the other a company doing no manufacturing but concerned merely with the distribution of stains. It was learned after the War that each of these organizations was selling a complete line of biological stains and the rival claims of the two added to the confusion of biologists.

To eliminate some of this confusion a committee was appointed by the National Research Council in 1921 which the following year established the Commission on Standardization of Biological Stains (usually known by the shorter title "Biological Stain Commission"). This Commission was established as an organization to coördinate the work on stains in which various American scientific organizations were interested. It was, accordingly, planned that each member of its Executive Committee should be a representative of one of these national societies and the Commission was regarded as deriving its authority from the appointment of these representatives

by their respective organizations. The national scientific societies which are thus represented at present are as follows:

Botanical Society of America
American Association of Anatomists
American Association of Pathologists and Bacteriologists
American Chemical Society
American Medical Association
American Public Health Association
American Society of Zoologists
Society of American Bacteriologists

The initial work of the Stain Commission was largely research, but as the investigations began to yield results showing the nature of the dyes best suited for particular biological purposes, such research has gradually been replaced by work of a more routine nature. A large part of its present activities are to test stains submitted by the manufacturers and to certify those found to be satisfactory.

For this purpose samples are submitted by the stain companies whenever a new batch of any stain has been manufactured. Ordinarily the company submitting the sample plans to send it in for testing sufficiently in advance of their needs so that the batch from which it is taken will not have to be put on sale before a report has been made upon it. Certification, in fact, is carried out entirely on a batch basis. No stain company has ever been given a blanket certification for its entire production of any particular dye. This plan of certifying each batch individually was adopted at the outset because chemical tests were known to be lacking that could properly distinguish between satisfactory and unsatisfactory samples. In many cases, in fact, it was realized that not even the most painstaking and conscientious manufacturer of dyes could be absolutely certain of duplicating exactly a satisfactory sample of a stain when it was time to prepare the next lot.

Two general types of tests are carried out on such samples: (1) physico-chemical; (2) biological. The physico-chemical tests which are made in the Color Laboratory of the Department of Agriculture are performed there by an associate employed by the Stain Commission who is located at Washington and does her work in the Color Laboratory; these tests are both chemical and optical and have been developed little by little in the course of the work as giving the most useful information in the case of each particular dye. The biological tests are more varied than the chemical tests

because comparatively few of the dyes are used by biologists in identical procedures, and this has made it necessary in many cases to test a stain by some method that is used for no other dye. It is considered particularly important, in fact, to determine the performance of each stain in the procedures for which it is ordinarily employed in the biological laboratory.

A detailed summary of the methods used in testing stains submitted for certification has recently been published by Peterson, Conn and Melin (18).

When the certification plan was first adopted, the samples submitted by the American stain companies were by no means uniformly satisfactory, but the quality steadily improved for the first four years, the percentage of rejected samples among those which were submitted for certification decreasing from 45 per cent in 1923 to 7 per cent in 1926. Since that date the number of samples rejected has averaged 8 per cent of the number submitted for certification. In two recent years, 1930 and 1935, certification was granted on every sample submitted for testing. This improvement in the supply of stains cannot be regarded in any sense as indicating a change of heart on the part of manufacturers, who have always been very anxious to put out reliable products, but to a better understanding on their part of the biologists' requirements. Their coöperation with users of stains during recent years has undoubtedly contributed to this better understanding.

Stains passing these tests are sold by the stain companies under a special certification label. The increased demand for certified stains during the period in which this work has been carried on is indicated by the following figures showing the number of certification labels furnished the companies during the past 12 years, divided into four-year periods:

1924-1927	46,530; average per year	11,633
1928-1931	60,560; " "	15,140
1932-1935	68,574; " "	17,144

It is interesting for purposes of comparison to note that during 1936 the certification labels furnished have amounted to 26,678.

RECENT DEVELOPMENTS

Counterstains

One seldom thinks of cytoplasmic stains as being of such critical importance as those designed to stain nuclei. The impression is

not uncommon that almost any acid dye which shows the right degree of contrast with the nuclear dye in use will be all right as a counterstain. Recent investigations, however, have shown that in many instances the correct choice of a counterstain can be fully as important as in the case of the nuclear stains.

Red Counterstains: A classic choice of a red counterstain is eosin Y which has been used for so long in contrast to methylene blue or hematoxylin that in many laboratories no other selection seems natural. It is only in comparatively recent years that much attention has been given by biologists to the higher homologs of the eosin group of dyes. Erythrosin, to be sure, has been known for some time although without coming into anywhere near as general use as eosin Y; but not until the last 15 years or so has much appeared in the literature concerning eosin B, phloxine or rose bengal. These dyes are more highly substituted compounds of the eosin series, eosin B being a nitro derivative, while the other two are more highly halogenated than eosin Y. The result of this introduction of nitro groups or halogen atoms is to deepen the shade, *i.e.*, shift it more toward the blue, and at the same time it seems to increase the selectivity of the dye and to decrease the ease with which it can be extracted from the structures stained.

One reason why these dyes have not been fully appreciated by biologists until recent years is because of confusion in identity. Thus Mallory (15) proposed an eosin-methylene-blue stain in which, contrary to the usual custom, the acid dye preceded the basic. Later he was unable to duplicate his results with American dyes until he found that his original eosin had not been eosin Y but either phloxine or something very much like it. At present he recommends phloxine. The logic of this is easy to understand. Eosin Y with its lower tinctorial power and greater ease of decolorization is satisfactory when it follows the basic dye, but is completely removed from the cytoplasm by the more powerful methylene blue when the acid dye is used first. Phloxine, which is a deeper and more powerful dye, is not removed by the methylene blue and, accordingly, can precede the latter.

This is not the only instance of mislabelling that has been detected in recent years; and one of the most striking illustrations, it happens, also involves phloxine. In a well known technic for staining algae, the old procedure called for magdala red, a very expensive

basic dye. It has proved, however, that magdala red actually has not been used for this purpose and will not work in the technic, as the acid dyes, phloxine and erythrosin, have masqueraded under this name and have actually been used in this method by botanists. This mislabelling was detected not very long ago, and now, in fact, Chamberlain (2) calls for phloxine instead of magdala red in this procedure.

Some years ago the writer (3) called attention to the value of rose bengal as a bacterial stain and later Conn and Holmes (5) made a fairly comprehensive study of all the compounds of this group available to determine which were the better bacterial stains. A subsequent investigation (6) showed that the staining properties of these dyes were greatly influenced by the nature of the anion with which they are combined, the calcium salts, for instance, proving more powerful stains than the more common sodium salts.

These investigations of the eosin group of dyes have in many instances pointed the way to a more satisfactory choice of a red counterstain.

A very different type of red counterstain is acid fuchsin. The chemistry of this dye has been well understood for a long time but it is such an indefinite mixture of compounds of different degrees of sulfonation that a uniform product is difficult to obtain. Scanlan, French and Holmes (19), however, showed a method of preparation which results in a considerably more uniform product than the older processes. The procedure proposed by these authors is now well known to American manufacturers and seems to have brought about a distinct improvement in their acid fuchsin.

Green Counterstains: The advantages of a green cytoplasmic stain when a red nuclear stain is employed have been appreciated by biologists for some time. Botanists especially have liked the green counterstains, although recognizing their limitations, especially in the matter of permanency. The most frequent choice has been light green, a name, by the way, which is rather indefinite since there are numerous light greens known in the dye industry. The correct name for the particular light green which seems to have been most used in histology is light green SF yellowish. Malachite green, also known as light green N, has sometimes been employed in similar procedures, but the general choice for a green counterstain is undoubtedly light green SF yellowish. Its shade and stain-

ing properties are greatly appreciated, but it readily fades and, accordingly, is not satisfactory for permanent preparations.

The greatest improvement in this situation occurred when attention was called to a new green dye, fast green FCF, which had recently been introduced as a food color and was proving to be less subject to fading than the usual green dyes. This dye was promptly tried by Haynes (10), proving such a satisfactory substitute for light green SF yellowish that it is rapidly supplanting it in histological and cytological procedures where a green counterstain is desired. Preparations stained with this dye have a very satisfactory degree of permanence.

NUCLEAR STAINS

On account of the great importance of the basic dyes employed as nuclear or chromatic stains they were the first to be investigated in the early '20's, and much of what was done at that time in the way of standardization is now an old story. There are, however, a few recent developments and certain other matters of progress in connection with such stains that are of sufficient importance to be repeated here even though the work was done sometime in the past.

Gentian Violet: The term "gentian violet," as has frequently been pointed out, is one whose use should logically be discontinued in favor of those employed in the dye industry for the dyes which enter into the composition of this stain, namely, crystal violet and methyl violet. Crystal violet is a definitely known chemical compound, hexamethylpararosanilin, while the different methyl violets are varying mixtures of this compound with others of lower methylation. Gentian violet, as interpreted by the Stain Commission, may be either crystal violet or one of the more highly methylated (*i.e.*, bluer) methyl violets. For nearly all purposes the use of crystal violet instead of gentian violet is now recommended, as it is a chemical entity. The only procedures calling for gentian violet in which crystal violet will not work are those in which the shade is too blue to contrast properly with some counterstain employed. In such cases it is recommended to use methyl violet 2B, and to order it as such rather than as gentian violet.

These recommendations were made fully 15 years ago and unquestionably more of the newer procedures call for crystal violet than was formerly the case. The stain companies, however, still request

that they be allowed to sell either methyl violet or crystal violet under the name of gentian violet because of the large number of orders for the latter which they receive. Accordingly, it seems to be important to continue repeating the recommendations against specifying gentian violet as such.

Safranin: Safranin is an important cytological stain but is used in such small quantities that the demand for it is not great. Accordingly, a batch prepared by a stain company sometimes lasts many years. In fact, only 18 batches of safranin have been submitted for certification by the three most important stain companies during the 12 years that this dye has been on the certification basis.

As a result of this small number of samples of safranin that have been available to test, progress in the standardization of this dye has been slow and nothing of a true scientific nature has been learned as to why some lots are satisfactory and some are not. It can be stated without question that the supply of stain available in America to-day is better than that of 15 years ago, but even yet an occasional batch seems to be unsatisfactory for some particular cytological procedure. It has not proved possible, however, to investigate thoroughly any such observations that have been made, partly because of the small number of samples of the dye available and also because cytological procedures are exacting and the personal equation is large. This makes it difficult for any two investigators to agree in their relative rating of any list of samples they compare.

Accordingly, there still undoubtedly remains considerable standardization work to be done and it will be greatly appreciated if anyone who finds a batch of this stain unsatisfactory in his technic will communicate with the Stain Commission.

Thiazin Dyes: The most important members of this group of dyes are thionin, methylene blue and toluidine blue. Chemically they are very well understood dyes and methylene blue was the first to be put on the certification basis. Almost every batch of this latter dye submitted for certification has proved satisfactory, although an occasionally less desirable sample still appears. Thionin is even better understood chemically than methylene blue. Although it is in far less demand than methylene blue, its metachromatic properties and characteristics make it an extremely useful histological stain. Its standardization has never presented any problem, though it was at one time confused with thionin blue which is a distinctly different dye.

The real problems in connection with the thiazin dyes have centered around the oxidation products of methylene blue and their relation to certain other dyes of the group, primarily toluidine blue. The first of these oxidation products to be described was Giemsa's azure I, whose composition was not known for some time and whose method of preparation still remains secret. Investigations were soon made, however, of its chemical nature by Bernthsen (1) and MacNeal (13, 14), the work of these investigations indicating that it was a mixture of two chemical compounds which, after their discovery, were named azure A and azure B. It was shown that these azures are intermediate in chemical composition between thioin and methylene blue. Later work by Holmes and French (12) suggested that there are even more possible chemical compounds in an oxidation product of methylene blue, such as azure I, than were realized by MacNeal; the most important new dye which they derived in this way they named azure C. The chemical findings of these later investigators have remained unchallenged and are assumed to be correct.

The use of these oxidation products of methylene blue as stains or components of stains has also received considerable investigation. The value of azure I in blood stains was well shown by Giemsa (9), while MacNeal (14) stressed the importance of azure A for staining purposes and particularly in place of azure I as a constituent of blood stains. To-day, in fact, azure A is ordinarily called for in America when making up a Giemsa stain or MacNeal's tetrachrome stain. The use of these azures as tissue stains has also been investigated, French (8) in particular calling attention to the value of azure C. Holmes and French (12) agreed with MacNeal that azure B was not a good stain. Haynes (11) disagreed with this and found with the proper technic equally good results could be obtained with azure A, B or C. Largely as a result of such investigations, azure A, which is the easiest of the three to prepare, is the only one at present supplied biologists in America.

Another question that has arisen recently in this connection is the possibility of substituting toluidine blue O for azure A. This is a textile dye that is very easy to prepare, whereas the only known method of manufacturing azure A is by the oxidation of methylene blue. Chemically, toluidine blue O is very similar to azure A, although produced from a different intermediate. It has been

found in some procedures that these two dyes give almost identical results; and very recent work, still unpublished, indicates that their absorption spectra are so nearly alike that it is very difficult to tell them apart by spectrophotometric tests. It is felt at present, accordingly, that investigations should be made to see how nearly toluidine blue can actually supplant azure A, since obviously the use of a more cheaply prepared dye would be desired if possible. Because of the relatively small demand for azure A, however, this problem is not pressing and no investigation of it has yet been made.

Methyl Green: Methyl green is typically a rather loosely formed compound of crystal violet with a methyl halide. Thus it has one more methyl group than crystal violet (*i.e.*, 7 instead of 6). As this compound is easily decomposed there is considerable possibility of any given sample containing a large proportion of crystal violet instead of being the green dye intended. This may well have been the reason for the numerous complaints made 10 to 15 years ago concerning the quality of the methyl green on the market.

Recently a slightly new type of methyl green has been submitted for certification which seems to be proving more uniformly satisfactory. Information available seems to indicate, however, that this new type of methyl green is really formed by combining crystal violet with an ethyl halide, as a result of which it must have one ethyl and 6 methyl groups instead of 7 methyl. The dye of this composition is usually called ethyl green, but as this name is also a synonym of brilliant green its use does not seem entirely desirable. As the product is almost identical in its behavior with the regular methyl green it is now being sold as methyl green and seems for this purpose to be giving general satisfaction.

Basic Fuchsin: Much recent investigation has been carried on concerning basic fuchsin, trying to learn what types are best suited for definite purposes. There are four different primary basic fuchsins possible, differing from one another in the number of tolyl *vs.* benzyl radicals in the molecule. Without going into the chemistry of the matter it can be said that these four primary compounds have been designated magenta O, magenta I, magenta II and magenta III. Ordinary basic fuchsins on the market are mixtures of magenta O and magenta I.

Now, basic fuchsin is used for a considerable variety of purposes: as a bacterial stain; occasionally as a histological stain; as a

chemical reagent for detecting aldehydes; as an indicator in the Endo medium used in bacteriology; and as a microchemical reagent in the Feulgen stain. The last three purposes all depend upon the property of this dye to decolorize in the presence of sulfite and of having its color restored, although to a rather more violet shade than that of typical fuchsin, in the presence of aldehyde or aldehyde-like substances.

It was thought for some time that the solution of the fuchsin problem might be that of controlling its chemical composition in seeing that the proper mixture of the four magentas above mentioned was present. Recent investigation, however, indicates that there is little correlation between this sort of variation in chemical composition and behavior for any of the above-mentioned purposes. It has proved very easy to secure a reliable basic fuchsin satisfactory for staining purposes, but the other three uses which depend on decolorization with sulfite and subsequent restoration of color have presented more serious problems (17, 4). The latest work on fuchsins for the Endo medium tend to lay the stress not on the chemical composition of the fuchsin but upon the proper combination of fuchsin and sulfite in the decolorized reagent. Although precautions of this kind help to solve the problem in the case of the Endo medium, it is recognized that most basic fuchsins now on the market contain some colored impurity that does not reduce in the presence of sulfite and hence interferes with proper behavior of this dye as a reagent for detecting aldehyde or as a microchemical reagent in the Feulgen stain. Work is at present in progress toward the removal of this impurity. The work is being done by the Industrial Farm Products Division, Bureau of Chemistry and Soils, Washington, D. C. (20), and it is believed that a solution of the problem is at hand. If this is true, a basic fuchsin more reliable and suitable for all purposes will shortly be commercially available.

In the meantime, the Feulgen technic, as well as the dye employed in it, has been undergoing improvement. Margolena (16) showed its value for bringing out certain cytological details in plant material; while most recently de Tomasi (7) has shown how to make it more reliable by certain changes in the technic and by the choice of proper counterstain. It is felt that when this procedure can be standardized and fully controlled, its value in cytological work will be quickly recognized.

CONCLUSIONS

In conclusion it can be stated confidently that the stains on the American market are of better quality and very much more uniform than they were 15 to 20 years ago—more so, in fact, than they were previous to 1914 when they were practically all purchased from one German company. There are two principal reasons for the distinct improvement that has been brought about: first, the eagerness of American biologists to take part in the necessary research and control work; and secondly, the wholehearted coöperation that has been secured from the stain producers in this country.

There is, however, room for improvement in two different respects. In the first place, an occasional sample of some well known stain is submitted that is not up to the usual quality. This is never because any company intends to put out an inferior product but rather because of the difficulty of standardizing perfectly such complex chemicals as dyes. It is to keep these occasional inferior lots off the market that continual inspection of the dyes still seems necessary.

In the second place, the biologists' demands in the case of stains are continually changing. As new procedures are developed or old methods perfected, new dyes are called for or more exacting demands are made upon the old dyes. In either case, investigation proves necessary in order to be sure that the stains on the market meet the new requirements. In other countries there is a very evident effort on the part of stain companies to exploit such newly discovered methods by putting on the market some new dye or dye mixture of secret formula which is intended to meet the new demand. Such efforts as this merely confuse the biologist and hardly work to his advantage. The constant effort in America, on the other hand, has been to keep the list of stains on the market as small as possible and not to confuse the purchaser with several names for the same dye or special designations for mixtures of well known dyes.

In short, the present situation seems to be quite favorable. Stains are becoming more and more standardized and little by little their use is being put on a more scientific basis. If the next 15 years show as much progress as the last 15, little should remain to be desired in either respect.

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THE BOTANICAL REVIEW

VOL. III

MARCH, 1937

No. 3

THE NITROGEN NUTRITION OF GREEN PLANTS*†

GORDON T. NIGHTINGALE

Pineapple Producers' Cooperative Association, Hawaii.

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* Nitrogen-fixation by leguminous plants is a specialized phase of nitrogen nutrition which is not reviewed here. There is likewise omitted any discussion of the interrelation between disease and nitrogen nutrition, and of responses to mineral deficiencies. For an excellent review of the theories of protein metabolism, consult Robinson (208). Especial attention should also be called to the hypotheses of the structure of proteins by Vickery and Osborne (271).

† Published with the approval of the Director as Miscellaneous Paper No. 21 of the Pineapple Experiment Station, University of Hawaii.

Plants absorb and utilize inorganic salts of nitrogen including ammonium, nitrite and nitrate with various degrees of efficiency. The relative rate at which plants can absorb and elaborate nitrogenous nutrients is dependent upon such external factors as the pH of the soil or nutrient solution, its concentration of solutes, and the relative availability of calcium, potassium, phosphate, etc. External factors such as light, temperature, moisture and oxygen supply also play an important rôle.

But internal factors, frequently given no consideration, are at least of equal if not of greater importance. Seeds, storage organs and growing plants vary enormously in their content and quality of nitrogenous and carbohydrate, or related nitrogen-free, reserves. The pioneer work on nitrogen nutrition, initiated nearly 40 years ago by Prianischnikov and his students (181, etc.), shows clearly that the quantity and nature of reserve materials in the plant profoundly influence nitrogen nutrition: the absorption and new synthesis of amino acids and associated materials from inorganic sources of nitrogen.

The subject of nitrogen nutrition in its several aspects to be discussed in the following pages can best be considered following a brief review of the metabolic changes which have been found to take place in organic compounds of nitrogen already in storage or contained in the plant. This phase of nitrogen metabolism is in contrast to new synthesis from inorganic nitrogenous salts. It is mainly concerned with the hydrolysis and regeneration of proteinaceous materials as they have been found to occur under various conditions of environment and carbohydrate supply.

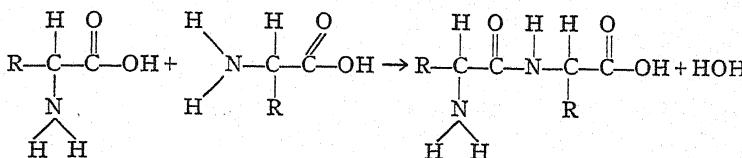
SYNTHESIS AND HYDROLYSIS OF STORAGE PROTEINS

There is available no satisfactory chemical basis for distinguishing between storage proteins and proteins of the protoplasm of active leaves or of growing or meristematic tissues such as cambium, root tip or stem tip. The former are found in tissues low in moisture and high in carbohydrates or their derivatives, in tubers and similar organs and in the cotyledons or endosperm of seeds. The latter are found in cells relatively high in moisture and lower in nitrogen-free materials.

Protoplasmic proteins have not been thoroughly investigated but it is well known that they do not give characteristic protein tests

except when preceded by denaturization. They occur apparently in a relatively latent condition and include complex nucleo-proteins which may in turn be cleavage products of more complex units. The cyclic structure hypothesis may perhaps lead to a better understanding of the nature of the protoplasmic proteins, for it indicates that amino acids can enter into combinations that are not included in the conventional peptide hypothesis (271).

Synthesis of Storage Proteins: The peptide hypothesis of Emil Fischer and others (271) is primarily one of synthesis of polypeptides by dehydration of amino acids. For every two molecules of amino acid that condense to form a more complex compound there is loss of a molecule of water thus :



This hypothesis is demonstrable in the laboratory and while it does not explain the synthesis of protoplasmic proteins of active plant tissues, it still remains one of the foundation stones of protein chemistry and, as will be seen in the following paragraphs, is in complete harmony with certain phases of protein metabolism in plants.

Schulze and his co-workers (230, 231) studied the chemical changes that occurred in the ripening of seeds of legumes. In the pea plant they found that the proteins of the pod were broken down to soluble organic compounds of nitrogen of which about one-half was asparagine and the remainder the amino acids tryptophane, histidine, leucine, and a small quantity of arginine. As the seeds ripened there was a decrease in the soluble nitrogen of the pods and a closely corresponding increase in the relatively complex amino acids and proteins of the seeds. In the completely ripe seeds there were found only extremely small quantities of asparagine, monamino acids, arginine and histidine. Wassilieff's (284) results indicate, however, that amino acids and asparagine do not always accumulate in the pod but may apparently be derived directly from stems and leaves. At least with accumulation of storage protein in the seed there was a marked decrease in the simpler soluble forms of organic nitrogen in all parts of the plant.

Zaleski (301) worked with the maturing seeds of pea which had been removed from the plant and he found that as the protein of the seeds increased with ripening there was a corresponding decrease in amino acids. Consistent results were not obtained in the case of all other species under similar treatment but this may well have been associated with removal of seeds prior to the deposition in the developing seed of an adequate carbohydrate reserve for respiration. Under such conditions, as will be shown presently, the dominant phase of protein metabolism is not of synthesis but of hydrolysis.

Many different kinds of seeds have been analyzed at various immature stages until completely ripe, and without exception the results have shown that as ripening progressed there was a rapid condensation of amino acids into polypeptides and storage proteins. (230, 303, 74, 17). In coffee and tobacco seeds there occurred with ripening not only dehydration of amino acids but also practically complete disappearance from the ripe seed of caffeine and nicotine, respectively. (75, 84). A discussion of the relationship of protein metabolism to xanthine derivatives and alkaloids is given by Weevers (286, 287). It has been found also by Klein and Taubock (100) that various immature seeds and fruits may contain free urea but that with maturation of the seed it can no longer be detected.

The synthesis of protein in ripening grain has been frequently studied (17, 21, 118, 119, 254, 297), various methods have been employed and the results are in general accord with those of Ecker-
son (56) who found no storage protein in the endosperm of the full grown, still green wheat kernel containing about 90% moisture. The aleurone layer and the layer of cells immediately below it contained more protoplasm than the other endosperm cells and gave a protein reaction; this, however, as she points out, was not storage protein. No storage protein was formed in the endosperm until desiccation began. From her tests it appears that the proteins gliadin and glutenin are formed when drying of the grain causes the amino acids in the endosperm to condense into proteins. She found no gluten until desiccation of the wheat grain began.

Grain, which when brought into the laboratory gave no protein reaction but contained much asparagine, arginine, histidine and leucine, was dried for 12 hours. It then gave a strong protein

reaction and contained gluten but had much less asparagine than before. On further desiccation, arginine, histidine and leucine disappeared and there remained only a trace of asparagine. The rôle of asparagine will be considered elsewhere but these results, with those of Schulze and others cited, are clearly in harmony with the peptide hypothesis of the synthesis of storage proteins by dehydration of amino acids.

It should be mentioned also that the chemical aspects of dehydration are not confined to amino acids (252). Monosaccharides condense to form disaccharides with loss of a molecule of water thus: $C_6H_{12}O_6$ plus $C_6H_{12}O_6$ equals $C_{12}H_{22}O_{11}$ plus H_2O . In the formation of starch ($C_6H_{10}O_5$)ⁿ or any other polysaccharide there is further dehydration. Both processes, the synthesis of storage proteins and the synthesis of reserve carbohydrates, are in harmony with the well known fact that seeds, storage organs and the plant as a whole decrease in moisture content as ripening or maturation progresses.

Hydrolysis of Storage Proteins: Whereas the synthesis of storage proteins as exemplified in the ripening of seeds involves the loss of water and is associated with carbohydrate accumulation, the breaking down or hydrolysis of storage proteins is correlated with the taking on of water and decrease in concentration of carbohydrates. Germination of seeds is one of the most common examples of a plant response in which proteolysis is a dominant phase of metabolism although of course accompanied, at least during early stages, by a gain in complex nucleo-proteins owing to increase in amount of meristematic tissue (166). A few illustrations may be cited but first it should be pointed out that in the plant, in addition to urea, two acid amides containing the characteristic $-CONH_2$ group have been found: asparagine, a semi-amide of amino-succinic acid ($COOH \cdot CHNH_2 \cdot CH_2 \cdot CONH_2$), and glutamine, a semi-amide of amino-glutaric acid ($HOOC \cdot NH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CONH_2$). Both are very common and one or the other or both are practically always present in higher plants (243, 135).

Schulze (220, etc.) permitted seeds of various legumes to germinate in darkness and made a remarkable series of isolations of many of the actual nitrogenous compounds concerned. Seedlings left in the dark for a week decreased greatly in storage protein owing to the formation by hydrolysis of the amino acids leucine, histidine,

lysine, tyrosine, and a small quantity of asparagine or glutamine. After the seedlings remained without light for two or three weeks and necessarily with accompanying decrease in carbohydrates, the amino acids mentioned decreased and there was a striking increase in amide. The form of amide varied with the kind of plant and possibly with the conditions of environment and the nature of the nitrogen-free reserves in the cotyledons (224). Both asparagine and glutamine were found in different proportions, one amide in some plants apparently almost completely replacing the other.

In the etiolated seedlings the decrease in amount of storage protein was relatively great if the seeds were low in their initial reserve of carbohydrates or fat. Likewise the decrease in amino acids and increase in amides was most pronounced under conditions of carbohydrate deficiency. For example, Schulze and Castoro (227) found that the amino acid content of etiolated seedlings one week old was much greater than that of comparable plants two weeks old growing in light, whereas amide nitrogen was about the same. It has been shown also that seedlings grown in the dark *with no external source of inorganic nitrogen* and supplied with sugar, form relatively little asparagine and ammonium (250, 251). Many additional experiments (87, 88, 136, 248), similar in nature, have since corroborated the earlier work of Schulze (220, etc.).

Results similar to those described were obtained by Prianischnikov (181). In fact, he found that asparagine accumulation continued even after protein hydrolysis had ceased and that this was accompanied by a diminution in concentration of amino acids and an increase in ammonium as carbohydrates became depleted in darkness. On the basis of their experimental evidence, Schulze and Prianischnikov eventually came to much the same conclusion. Their results show that it is essential to distinguish between the hydrolytic splitting of storage protein by proteolytic enzymes and a further oxidation change of the cleavage products. The former primary process yields amino acids, organic bases and small quantities of amides, but probably only insofar as they were preformed in the disintegrated protein.* Portions of these primary hydrolytic products, apparently especially the bases alanin and leucin (68, 228), undergo oxidation with the formation of ammonia.

* Of course, it may be possible, as suggested by Schulze and others, that some ammonia formed in plants may be split off directly from the protein molecule and with the simultaneous presence of malic acid in germinating seeds there could well occur the union of this acid with ammonia to form asparagine.

The investigations of Godlewski (68) furnish additional proof of the secondary origin of amides, in that he was able to show that germinating seeds in oxygen-free air formed amino acids, whereas asparagine and ammonium, which normally appeared, were not present in measurable quantity. Likewise Butkewitch (26), who successfully demonstrated the presence of proteolytic enzymes in seeds, was able to show that in anaesthetized seedlings no asparagine was formed even though ammonium was formed (*cf.* 117, 249, 284, 135, 300).

Prianischnikov especially has compared the rôle of ammonium and amides in plants with urea formation in animals (189). This immediate discussion, while concerned with the internal formation of ammonia, may be compared with Prianischnikov's work to be considered in greater detail later. He was concerned with the formation of asparagine from ammonium derived from external as well as internal sources. However, other conditions being equal, there seems not the slightest reason to think that the origin of ammonia would in any way modify its subsequent metabolism within the plant.

Prianischnikov's views, which have apparently become rather generally accepted, are summarized by Murneek (146). With a decrease in carbohydrates, amino acids are formed through hydrolysis of proteins by means of proteolytic enzymes. With considerable depletion of available carbohydrates it appears that amino acids are oxidized and amino groups released. Asparagine, containing two amino groups, is considered to be formed from two molecules of amino acids, one of these being oxidized to aspartic acid, the other much further, with splitting off of ammonia. There is presumed to be then a union of aspartic acid with ammonia to form ammonium aspartate, from which, through dehydration, asparagine would be produced in the same manner as, in the animal organism, urea is formed from carbamate of ammonia. When a plant containing an adequate carbohydrate reserve is supplied with an abundance or slight excess of ammonium, asparagine may presumably be formed from one molecule of an amino acid or even from an organic acid such as malic or succinic acid. With growth of the plant and development of new tissues, asparagine may apparently be broken down, ammonia released, and employed for amino acid synthesis and subsequent protein formation. Thus asparagine and

likewise glutamine are said to function in removal of injurious ammonia and in storage of nitrogen (*cf. Detoxication of Ammonia*). Studies by Klein and his coworkers (99, 100, 103, 104) show that urea is present in some green plants and seems to be in many respects similar to asparagine and glutamine in function. Urease (93, 94, 96, 175) has been found in plants as well as asparaginase (71), arginase (102) and other enzymes apparently associated with protein metabolism (26, 280, 296).

Storage Organs: It would not seem essential to discuss in detail metabolic changes occurring in the nitrogenous compounds of storage organs such as corms, bulbs, rhizomes and storage roots. In general, they follow the same course of metabolism as seeds. Associated with much higher percentages of moisture in storage structures, there is invariably found, as would be expected, a relatively high proportion of soluble organic nitrogen and a proportionately lower concentration of protein than in seeds where ripening has been shown to be characterized by desiccation and the synthesis of protein at the expense of amino acids. It should also be recalled that many storage organs contain a comparatively high proportion of meristematic tissue such as the supernumerary cambiums of the storage root of beet (7) or sweet potato (6) and the specialized dividing cells of the tubers of the white potato (5). The proteins of such cells can scarcely be considered storage proteins.

It is not surprising, therefore, that studies of metabolic changes of entire storage organs have sometimes yielded less precise results than investigations of seeds. Grüntrich (72) has reviewed much of the earlier work concerning storage organs and has conducted an extensive series of experiments with various underground reserve structures of many plants. Unfortunately his analyses do not differentiate between organic and inorganic nitrogen. Nitrate was not determined, yet some of the plants he worked with store nitrate in high concentrations. However, where nitrate has been determined and where it has been possible to separate storage tissues, as the older storage scales of bulbs, from the central relatively meristematic tissues, responses have been obtained which corresponded closely to those already recorded for seeds (153, 200); that is, sprouting of storage organs in darkness is associated with a decrease in carbohydrates in strictly storage cells, hydrolysis of proteins to amino acids and the later appearance of amides very much as in seeds

(220, etc., 299, 151, 247). In general, as in seeds, the higher the concentration of carbohydrates the less drastic the proteolytic changes. In fact, dormant onion bulbs notably high in sugars and amino acids but low in amides may during sprouting go so far as to exhibit protein regeneration from amino acids in darkness, although in part of a non-storage type, if the period of etiolation is not unduly prolonged (302). In accord with the results just cited and the previously described work of Schulze, Stuart and Appleman (247) make the significant observation that in the process of development of the meristematic cells involved in wound periderm formation in potatoes, the synthesis of the proteins concerned was associated with utilization of amino- rather than amide nitrogen. This response would seem particularly notable in view of the fact that amides are high in potato tubers. Obviously these results support the theory of protein regeneration from amino acids rather than from amides.

SYNTHESIS AND HYDROLYSIS OF LEAF PROTEINS

Assuming that both storage and protoplasmic proteins occur in leaves that are not of a specific storage type, there is no information making it possible to differentiate between them. In studies of progressive changes in leaf metabolism the proteins have in most cases been estimated on the basis of the total nitrogen content of a heterogeneous coagulum rather than as individual proteins. Various protein materials have been obtained, however, from leaves and compared as to their isoelectric point and hydrolytic products with similar preparations from other organs (49, 95, 165).

Chibnall (29, 31, 33) and Chibnall and Grover (34) indicate that there is a protein fraction in bean leaves considered to be cytoplasmic protein that, while varying in amount in young and old leaves and under different external conditions, nevertheless seems to remain constantly of about the same quality. Thomas (256) likewise found that the preparation which he designates as cytoplasmic protein of apple leaves varied in amount but was approximately the same in amino acid constitution regardless of the stage of development of the leaf. On the other hand, Vickery *et al.* (274) reported that somewhat more than half of the protein of the tobacco leaf was less stable to the enzymes of the cells than the remainder, which they suggest may be in the nature of a reserve protein. The

methods of extracting the leaf proteins are of course described in the several cases but it remains, so far, uncertain as to the exact part or parts of the leaf protoplasmic mass contributing to the protein preparations.

Ullrich's (269) results are of interest in this connection since on the basis of microchemical and cytological examinations he observed a close relationship between the size of the chloroplasts and the protein content of the leaf. Meyer (133) likewise noted that as leaves became older or etiolated there was the usual decrease in green color, relatively little change in cytoplasm or nucleus, but a marked decrease in size of the chloroplasts that was correlated with disappearance of protein from the leaf.

Metabolism of Attached Leaves: Schulze and Schutz (229) followed the diurnal changes in leaves from two box elder trees and found that there was a loss of nitrogen from the leaves at night. This was correlated with a decrease in carbohydrates and protein whereas the soluble organic fractions of nitrogen changed little. Bean leaves also lost in protein content at night, according to Chibnall (31). He ascribes this to the breaking down of cytoplasmic proteins. In these experiments, however, as in similar cases (10, 138), there was furnished little evidence as to the immediate products of protein hydrolysis because they were apparently translocated from the leaf. As leaves of trees mature or as the lower leaves of herbaceous plants approach senescence there is, before leaf-fall, a loss of protein and a gain in nitrogenous material in other organs (41, 52, 138, 145, 173, 229, 257). If nitrogen is deficient there is decrease in protein in the lower leaves and migration of nitrogen from these organs to younger tissues, as shown by Richards and Templeton (206), Mason and Maskel (120, etc.) and others (62, 135). This process, of course, involves protein cleavage and translocation of the hydrolytic products. Proteolysis in leaves, as in other organs, may be greatly accelerated in rate if nitrogen deficient plants, usually high in carbohydrates, are shifted to darkness, thereby decreasing their carbohydrate reserves (154). Here again detailed information concerning the nature of the metabolic changes is obviously made uncertain owing to translocation. Accordingly detached leaves have frequently been investigated.

Metabolism of Detached Leaves: The objection is often raised that in experiments with detached leaves abnormal conditions are

created. This is obvious and in fact the primary object in such investigations is to avoid normal translocation losses of nitrogenous bodies and thereby have such materials available for study. Although caution must necessarily be employed in interpretation it should be pointed out that work with excised leaves has supplied information of value that might not otherwise have been obtained. It may be pertinent to mention here that Mothes (135) found that when the petioles of detached leaves were immersed in water there was no significant loss of nitrogen from the cut ends. It should be said, however, that he took special precautions, supplying sterile water with frequent changes, and at least minimized the chances of contamination with microorganisms by washing the leaf surfaces with 1 to 2.5 per cent hydrogen peroxide, taking special care to employ leaf blades that were intact.

His work and that of others (150, 169, 170) indicate that there is a remarkable similarity between the nitrogenous metabolism of leaves and seedlings. This is especially evident in the so-called "protein-sparing" action of carbohydrates. For example, Deleano (50), working with detached shaded grape leaves with their petioles in water, found that respiration during the first three days went on only at the expense of carbohydrates. After that time, when a large part of the carbohydrate supply was exhausted and starches had practically disappeared, proteolysis began and after several days in darkness was accompanied by a considerable increase in ammonium. The total nitrogen content did not change appreciably. Spoehr and McGee (241) studied the responses of excised sunflower leaves in darkness and obtained a striking increase in amino acids when the petioles were in water only, whereas similar leaves with their petioles in a sugar solution exhibited practically no increase in amino nitrogen. The rate of hydrolysis of protein of grape leaves also was greatly reduced when the detached leaves were supplied with glucose (50).

Chibnall (29, 32) found that the attached leaves of the runner bean decreased materially in amount of protein at night although the quality, as already stated, apparently remained practically unchanged. In a parallel experiment using detached leaves with their petioles in water, he found that the decrease in protein occurring in darkness was associated with an increase in amino acids and asparagine.

The apparent secondary origin of asparagine and glutamine in seedlings has already been discussed and it seems reasonably clear that amide nitrogen in leaves also is formed through the oxidation of amino acids. Mothes (135) showed by several experiments with detached bean leaves, receiving no external supply of nitrogen, that amides began to appear in quantity only after 2 to 4 days in darkness, hydrolysis of proteins to amino acids apparently predominating during the early stages.

As further proof of the origin of amide nitrogen there are available the effects of anaesthesia. Mothes (135) placed the petioles of detached bean leaves in water in darkness under 8 liter flasks in which was evaporated .5 cc. of chloroform. This concentration was not apparently injurious to the leaves as some of them, returned to normal air conditions subsequent to treatment, followed the usual course of metabolism. These experiments and others with oxygen-free air indicated that oxygen was essential for amide formation. In anaesthetized high-carbohydrate leaves, not too old, neither amide nor ammonia appeared but apparently proteolysis to amino acids occurred unhindered in darkness. When similar leaves low in carbohydrates were employed, proteolytic activities were greater; ammonium accumulated but there was no formation of amide.

Mothes' (135, 137) results fully corroborated the earlier work of Schulze and Prianischnikov with seedlings. In addition, he found that old leaves, as the primary leaves of bean even before they showed any external signs of senescence, exhibited proteolysis and amide formation both on increase of carbohydrates in the light and on addition of glucose in the dark. This was not owing to exceptionally rapid catabolism but rather to limited synthesis. Leaves which had but recently become fully expanded were much more active proteolytically on decrease in carbohydrates in the dark. These results, in harmony with those of Everingham and Pearsall (64), emphasize the necessity of care in selecting plant material.

In brief, excepting "old leaves," Mothes (135) found that when there was opportunity for carbohydrate synthesis in sunlight or the leaves had an available supply of glucose in darkness, protein synthesis rather than hydrolysis predominated and there was a decrease rather than an increase in amides. The higher the temperature, however, the higher the concentration of glucose which had to be supplied to check proteolysis. On the other hand, when carbohy-

drate reserves became reduced through exposure to darkness there occurred first proteolysis and with further decrease in carbohydrates an increase in amides. Finally, ammonia accumulated with eventual injury to the leaf under conditions of extreme carbohydrate deficiency. In the presence of abundant carbohydrates in darkness or in light, ammonia was used in the formation as asparagine. Mothes (135) found that this was true whether the ammonia was derived externally from ammonium salts absorbed by the cut petiole or internally by proteolytic action. Whether or not there was direct synthesis of amino acids from ammonia was not made clear. However, amides entered directly or indirectly into the synthesis of proteins in darkness or in light if there was available an abundant carbohydrate supply. It made no difference whether the amide nitrogen was supplied from external sources, as asparagine, or was derived internally through the oxidation of amino acids.

In this connection Wood's (296) studies of the pH of leaf sap and accompanying proteolytic activity should be recorded even though the lack of pH data in Mothes' (135) experiments and the lack of carbohydrate determinations in those of Wood make suggestions of interrelations purely speculative. Wood noted that the pH values of the leaves of *Atriplex nummularium* were intimately associated with enzymatic activity. He obtained leaves of different degrees of acidity by employing old and young leaves, by allowing plants to wilt and by supplying the petioles of excised leaves with acidulated water. When the sap was below pH 5.5 amino acids predominated; when less acid, there was an increase in amide plus ammonia. Extracts of leaves adjusted with a phosphate buffer to various pH values and subjected to autolysis, gave comparable results.

This matter will be considered again in connection with the ammonium and nitrate nutrition of plants of different degrees of acidity. Fife and Frampton's (65) results may, however, be cited. They decreased the acidity of the sap of beet plants and detached leaves by placing their plant material in an atmosphere high in carbon dioxide. In an hour or less there was a decrease in amide and a comparable increase in ammonium. A return of the plants or detached leaves to normal atmosphere resulted in a prompt reversal of the reaction, the amide increasing with a decrease in ammonium.

Vickery and Pucher (274) obtained results which are in part similar to those of Mothes (135). They followed the chemical changes that occurred in mature detached leaves of tobacco that were placed with their petioles in distilled water. Some of the leaves were analyzed at the beginning of the experiment and others at intervals up to 303 hours. Decrease in carbohydrates in diffuse light was accompanied by a slow decrease in nicotine and by protein hydrolysis. It is significant that a gain in amides was correlated with a decrease in amino acids.

They also record a result that seems to be without precedent and for which no explanation is available and for which they offer none. There apparently occurred during the early period of the experiments, while the leaves were still turgid, an increase in nitrate in the detached leaves which, it will be recalled, had no external nitrogen supply. This was soon followed by an apparent reduction of nitrate to ammonia. Mothes (135), peculiarly enough, in all his extensive work with excised leaves, for the most part obtained from soil-grown plants, gives no records as to the presence or absence of nitrate. Spoehr and McGee (241), in comparable experiments, state that special tests were made to determine whether there was an accumulation of nitrate in the leaves of sunflower which had been kept in the dark. However, no indication could be found that this was the case; in fact, no tests for nitrate could be obtained in the water extracts. The writer has frequently placed in darkness tomato plants that had no external supply of nitrogen and no nitrate in their tissues; in no case, regardless of the period of proteolysis in darkness, was a positive test for nitrate obtained. As Vickery *et al.* point out, further investigation will be required before an explanation can be offered as to the apparent synthesis of nitrate from organic nitrogenous materials already in storage in the plant.

Vickery and Pucher (272), in following chemical changes in slowly drying (curing) tobacco leaves, noted that during the period of wilting proteolysis was much more rapid than during a comparable period of time in the case of turgid detached leaves supplied with distilled water (277). Mothes (138) and Wood (296) also found that wilting materially increased the rate of protein hydrolysis.

METABOLISM OF STEMS

Aerial stems vary greatly in structure and function and in their rôle in nitrogen metabolism. Some types of stems are concerned

primarily with translocation, some with translocation and storage and others with both these functions and new synthesis of organic nitrogen as well (57, 60, 153, 154). Stems being less specialized in function than seeds, storage organs and leaves, have lent themselves less readily to studies of protein metabolism. Nevertheless, there is considerable evidence to show that in nitrogen economy they follow trends similar to those already recorded for other organs.

An accumulation of carbohydrates usually occurs under favorable light conditions in plants lacking an abundant external supply of nitrogen. With the onset of senescence there is undoubtedly a breakdown of stem protein (154), regardless of carbohydrate reserves, probably much as Mothes (135) reports for "old leaves". At high temperature in plant stems, as in leaves (135), proteolytic action takes place even in the presence of a high concentration of carbohydrates, although much less rapidly than in a low-carbohydrate plant (158, 160, 161). However, a high carbohydrate reserve is very frequently associated with condensation of amino acids to polypeptides and complex proteins, probably in part of a non-meristematic or storage type (153, etc.). At least under such conditions in tomato few meristematic cells are present except at the apex of the stem and roots.

Richards and Templeton (206) take exception to this view on the basis that under conditions of shortage of nitrogen the plants should not be expected to store the limiting element. Although this is a convenient hypothesis it means little. Their work, on the other hand, and that of Mason and Maskel (124, 125) are convincing. It indicates clearly that under conditions of nitrogen deficiency there is a definite migration of nitrogen from relatively mature or senescent tissues to meristematic zones. It should be emphasized, however, that this migration of nitrogen to meristematic tissues can be greatly accelerated and increased in amount by shifting high-carbohydrate, nitrogen-deficient plants to darkness (153, 154, 155, 157). Under these conditions there is a very rapid increase in volume of new tissue of stem and leaves that may very well be initiated by growth-substances or hormones, the gross chemical changes being possibly secondary in nature (*cf.* 289, 198). Nevertheless, this phenomenon is accompanied by a decrease in carbohydrates and there does occur protein hydrolysis much as in seeds, storage organs

and leaves. As long as some carbohydrates are available there is very rapid regeneration of the proteolytic products to form new proteins of the meristem (152, 154, 155). Concerning these proteins there is little known except that, as already pointed out, they are in contrast to storage proteins which are apparently most readily synthesized under conditions of high dry matter content; that is, a high concentration of carbohydrates or other nitrogen-free reserves. Further evidence in this direction is furnished by the chemical composition and growth responses of tomato plants and apple trees grown with no external nitrogen supply at high and low relative humidity. Low humidity clearly favored condensation of amino acids to proteins and little development of meristematic tissue; high humidity, the reverse (162) (*cf.* 128).

There has recently been accomplished some excellent work on the seasonal changes in the nitrogenous materials of trees (90, 141, 142, 143, 145, 173, 239, 257, 258, 260). Reference should also be made to earlier work cited by Chandler (28) and Gardner, Bradford and Hooker (67). This type of work has been carried out under different circumstances of environment and soil conditions, and different kinds and varieties of trees have been employed; therefore, a coherent adequate account of this work showing the proper interrelations cannot be readily included in the space allotted to this review.

The autumnal migration of nitrogen from leaves has already been mentioned in connection with the metabolism of these organs. This phenomenon seems well established and in general it also appears from the work cited above that in the late winter and early spring soluble organic compounds of nitrogen accumulate and are translocated to the developing buds and shoots. The probable origin of these materials will be considered later, especially in their relationship to the metabolism of roots. In some cases it appears that amino acids or polypeptides have been primarily concerned in transport; in other cases, amides.

It is generally considered that the soluble or crystalloid forms of nitrogen are concerned in translocation. Many references might be cited favoring a particular form of nitrogen but there is no data available at present which makes it possible to point out one form of soluble nitrogen found in plants as being of more importance than another in translocation (31, 32, 40, 51, 62, 114, 172, 208, 257).

For a consideration of the possible channels of nitrogen transport the reader is referred to the work of Maskel, Mason and their collaborators (120, etc.) and to a review by Curtis (44). It would seem reasonable to think, however, that the metabolic activities of the plant and the seat of initial organic nitrogen synthesis to be considered later, must in a considerable degree determine the form of nitrogen available for translocation. A material must certainly be present before it can be translocated and there is abundant evidence, to be considered in the following pages, indicating that with shift in environment or nutrient supply one or another form of nitrogen may predominate. For example, asparagine may be almost completely replaced by glutamine and the ratio of amide to amino nitrogen may be greatly changed depending simply upon whether the external nitrogen source is from an ammonium or nitrate salt (39).

THE NEW SYNTHESIS OF ORGANIC NITROGEN FROM NITROGENOUS NUTRIENTS

The metabolic changes that take place in organic compounds of nitrogen stored or contained in the plant have been considered in the preceding pages. The quantity and quality of these materials and carbohydrate reserves present and their relation to new synthesis of organic nitrogen from inorganic nitrogenous salts will next be discussed along with external factors influencing this process.

Absorption is one of the first stages in any phase of plant nutrition and, although closely associated with the general topic under consideration, will not be discussed in detail (*cf.* 79, 180, 261, 262). It may be well to recall, however, that living root cells can absorb solutes from very dilute nutrient or soil solutions. They can with extraordinary rapidity accumulate much higher concentrations of salts in their cells than in the surrounding nutrient solution. Granted an adequate growing root system in the presence of some free oxygen and a complete nutrient solution that permits salt dissociation, absorption or permeability of non-injured root cells seems seldom if ever to be a serious limiting factor in nitrogen nutrition. Failure of a plant to remove nitrate continually, for example, from a nutrient solution is, of course, failure to absorb but it seems, at least in the cases where plant analyses have been made, to be associated with capacity accumulation of nitrate within the plant rather than with impermeability of the absorbing cells of the root system.

If internal and external conditions are so modified as to permit reduction of the contained nitrate and new synthesis of amino acids, absorption is again renewed (37, 38, 47, 48, 233, 242).

As will be brought out in the following discussions, there is obviously no one best nutrient solution (110). The nutrient solution will necessarily vary with the experimental objectives, with the quality of plant growth desired, with the opportunity for carbon dioxide assimilation, with the initial reserves in the seed or other organ of propagation and with the stage of growth of the plant. Certain precautions are, however, absolutely essential. The pH of the culture or soil solution is of major importance, as has been pointed out repeatedly by Prianischnikov (181, etc.), Shive and his students (37, 38, 47, 48, 233, 242) and by many others. Methods of pH control of culture solutions have been given by Shive and Stahl (234), Trelease (267, 268), and Zinzadze (305). Many workers, however, have given no consideration to, or at least no report of, the pH of the soil or nutrient solution employed in their studies of nitrogen nutrition.

A commonly ignored factor in water culture experiments is the oxygen supply available to the immersed root system. For most non-aquatic plants aeration is absolutely essential. Shive (235) reports that when tomato plants were grown in culture solutions that were continuously aerated the average total yields of plants were more than 50 per cent higher than those of plants grown in non-aerated cultures. Determinations of the oxygen content of the culture solutions, both before and after the plants had grown in them for six hours, showed that even with continuous aeration the roots of the plants used up in a short time the greater portion of the oxygen which was presented in the culture solution before the plants were placed in them. The aerated solutions contained four to five times as much oxygen as did the non-aerated solutions. Plants grown in aerated cultures removed up to 60 per cent more nitrate and ammonium nitrogen from the cultures than did those grown in non-aerated cultures; the influence of aeration on ammonium nitrogen absorption rates was much more pronounced than on the nitrate absorption rates. Low nitrogen absorption rates and retarded growth rates in non-aerated cultures were caused by a deficiency of oxygen and not by an accumulation of carbon dioxide through excretion from the plant roots.

From these responses it is obvious that the metabolism of the plant is greatly modified when the nutrient lacks oxygen. Loehwing (111) grew sunflower and soy bean plants in aerated and non-aerated soil. His plants lacking aeration were soft, succulent and poorly developed mechanically in that lignification was limited. This was associated with carbohydrate deficiency and late flowering. Additional references might be cited (*cf.* 111, 171) but it will be sufficient to recall that, in addition to effects on carbohydrate reserves, lack of oxygen materially modifies protein metabolism, the dominant phase being hydrolysis rather than synthesis, not only in aerial organs (26, 135, 139) but in roots as well (14, 79).

Unfortunately, many experiments on nitrogen nutrition have been conducted in water cultures with no provision for aeration and in sand, soil and water culture with no record or no control of the pH value of the nutrient medium. There are cases where methods of plant analysis do not permit conclusions, where attempts have been made to estimate amide nitrogen by hydrolysis with hydrochloric acid in the presence of nitrate, where nitrate has been recorded as the difference between the Kjeldahl and the modified Kjeldahl procedure, where ammonium in plant tissues has been recorded as the amount of ammonia yielded after boiling plant extracts with sodium hydroxide, etc. (*cf.* 30, 35, 246, 273, 285).

Because the results of such work seem to the writer to be impossible of accurate interpretation, they are in general omitted in whole or in part from the following discussions without further reference. A useful and comparatively complete list of experiments on ammonium and nitrate nutrition has been recently made available by Pardo (168). As intended, it is primarily an annotated bibliography in which experimental results have been catalogued according to the plant family concerned.

STORAGE AND ASSIMILATION OF NITRATE

Nitrate Storage: Nitrate is apparently freely absorbed by uninjured roots (*cf. The pH of the Nutrient Solution*) over a wide range of pH values of a complete nutrient or soil solution and it may accumulate in the plant in enormous quantities without injury. Woo (295) reported that in the stems of *Amaranthus* 56 per cent of the total nitrogen was nitrate and Campbell (27) obtained similar results. Even in storage organs, as the roots of mangold, nitrate

has been found in high concentrations. In celery plants Platenius (179) recovered as high as 80 per cent of the soluble nitrogen as nitrate; in the vegetative organs of wheat McCalla (118) obtained, as the plants were maturing, 50 per cent of the total nitrogen was nitrate and in the oat plant Sessions and Shive (233) found that one-third of the total nitrogen was in this form. Chibnall and Miller (35) obtained high yields of nitrate from the leaves of rye grass, Vickery *et al.* (274) and Eisenmenger (61) from the tobacco plant, and many more references might be cited showing that nitrate accumulation commonly occurs in a wide variety of plants (43, 149).

Nitrate Reduction: The amount of nitrate stored and the organs concerned vary greatly with the kind of plant (55). In some plants, as tomato, if the sole external source of nitrogen is nitrate, a fairly high concentration must continually be maintained in the vegetative tissues to insure a synthesis of organic nitrogen from nitrate that is sufficiently rapid to maintain vigorous vegetative growth (105, 154, 265, 39). On the other hand, with ammonium as the sole external source of nitrogen, vigorous growth of tomato and many other plants may be maintained even though the plants concerned are entirely or practically free of nitrate (47, 48, 194, 197, 263, 265). Nitrate may, however, accumulate in tomato and other plants during a period of little growth with no apparent external effect on the plant (55, 58, 60, 152, 158). Any cell constituent may well be considered as exerting some effect on the development of the plant but it is obvious that nitrate is not a form of nitrogen essential for plant growth; it rather represents nutrient material not yet metabolized, a potential source of organic nitrogen.

There are many theories, for the most part purely speculative, concerning the processes involved in the transformation of nitrate to organic nitrogen. Robinson has contributed an excellent review partly on this subject (208). Knowledge of organic nitrogen synthesis from nitrate is far from complete but there is abundant evidence that the initial phases of the process include the reduction of nitrate to nitrite and ammonia. It has been reported that hydroxylamine is present in the leaves of certain plants in minute quantities, supposedly being formed following the appearance of nitrite, but at present any discussion concerning its possible significance would be purely speculative (108). It would be of interest, however, to know whether or not nitrate-free ammonium-supplied plants ever contain hydroxylamine.

Nitrate reduction will take place in plant cells in the dark (*cf. Nitrate Reduction in Roots*), although light is of course necessary in carbohydrate synthesis. The reduction of nitrate is a definitely endothermic reaction, however, and there cannot be said to be unequivocal proof that there may not be in nitrate reduction in some plants direct utilization of the energy of light through transformation to chemical energy. Nevertheless, in cases where carbohydrate analyses have been made, the reduction of nitrate in light as well as in darkness has been found to be accompanied by oxidation of sugars or their derivatives and a decrease of reserve carbohydrates in the organs concerned (57, etc., 257, 258, 259, 153, etc., 264, 265, 195, 47, 48, 55, 118, 203).

It is most important to appreciate the significance of this process in relation to carbohydrates in any consideration of nitrate nutrition. Eckerson (57) has followed the transformation of nitrate in tomato plants and has also recorded the associated changes in carbohydrate reserves. Her plants, after an initial period in soil, were grown for several weeks in quartz sand with no external supply of nitrogen. At the end of that time they exhibited typical symptoms of lack of nitrogen. They contained an abundance of glucose, some fructose, a little sucrose and a high concentration of starch in stems and leaves. The plants were free of nitrate, nitrite and ammonium and practically no amino acids could be detected. Some of the plants in this condition were supplied with calcium nitrate; in 24 hours all parts of the plant contained abundant nitrate and the tops of some of the plants gave a slight reaction for nitrite but no ammonium was found. Twelve hours later all the plants had considerable nitrite localized in the cortical cells of the stem tips and especially in the phloem region of the stem. After 48 hours there was slightly less nitrite but more ammonium. It is notable that a decrease of starch occurred in the tissues in which nitrite was observed. These responses were followed by the appearance of amino acids. However, in vigorously growing plants continually supplied with nitrate there seldom may be detected more than traces of nitrite and usually none is found. This is in accord with the results of many workers who have reported the presence of traces of nitrite in different kinds of plants (155, etc., 9, 55, 73, 86, 217, 259, 285, 108).

In connection with student instruction during the past few years the writer has frequently followed in tomato plants reactions essen-

tially the same as those recorded by Eckerson. Apparently the same series of chemical reactions have been obtained in varied experiments with other plants, some of which are to be described later. The reduction of nitrate to nitrite may be easily and consistently demonstrated in tomato by employing plants that are low in nitrogen but high in carbohydrate reserves and supplying them with abundant nitrate under favorable growing conditions. As a rule, the lower the nitrogen content of the plants, when coupled with high carbohydrate reserves, the more rapid the absorption and reduction of nitrate. Often two or three hours after application, nitrate can be found in abundance in all parts of a tomato plant and frequently nitrite will appear two or three hours later. In plants considerably higher in organic nitrogen and lower in carbohydrates, but likewise lacking nitrate, 12 hours or more are required for a comparable absorption response and two days may elapse before the appearance of nitrite. Dittrich's (55) results showed similar correlations between nitrogen concentration, carbohydrate content and reducase activity.

Hamner (73) worked with tomato plants in sand culture which were very similar in quality of growth to those employed by Eckerson. In a typical experiment his high-carbohydrate plants lacking nitrate were shifted from a greenhouse to a chamber maintained at a practically constant temperature of 22° C. and a relative humidity of 75 per cent. In alternate periods the plants were in darkness for 14 hours, and for 10 hours were exposed to light from tungsten lamps averaging about 850 foot candles at the surface of leaves which were enclosed in a chamber employed for measuring carbon dioxide exchange. The concentration of carbon dioxide in the air entering the leaf chamber was the same as in outside air. There was no evidence in this work that addition of nitrate to the tomato plants increased the rate of photosynthesis. As Hamner points out, this may have been owing to the light source, which compared unfavorably with that of sunlight in its effect on tomato, and to the fact that carbon dioxide may have been a limiting factor.

His results on respiration are of remarkable interest. Some of his plants, lacking an external supply of nitrate and containing none in their tissues, were supplied with nitrate *at the beginning of the period of darkness*. Nitrate, as part of a complete nutrient solution containing no ammonium, was added to the quartz sand of the

culture jars in the form of calcium nitrate. In four to six hours after adding this salt, tests for nitrate were positive well up in the plants. Nitrite became apparent two hours after nitrate was detected in the tops and shortly thereafter a striking increase in rate of respiration of carbon dioxide was observed as compared to comparable plants lacking nitrate.

His several different series of experimental plants were different in degree of nitrogen deficiency and carbohydrate content and, therefore, as would be anticipated, differences in the rate of nitrate absorption and reduction occurred. To be emphasized, however, are the facts that (1) he obtained reduction of nitrate to nitrite in darkness and (2) that in all cases the increase in rate of respiration occurred not with the initial appearance of nitrate in the plant but accompanying or following the reduction of nitrate to nitrite. Undoubtedly this was accompanied by the appearance of amino acids, as described by Eckerson, for his plants were definitely darker green 24 hours after receiving nitrate. In his series of plants which were comparatively high in carbohydrates this increase in respiration rate was of the order of 300 per cent; in plants lower in carbohydrates it was about 100 per cent. In wheat Hamner found that the responses in respiration under comparable conditions were very much the same as in tomato. This was true not only for the tops but for the roots of the wheat plant (*cf. Nitrate Reduction in Roots*). Further, Gregory and Richards (70) report that barley lacking abundant nitrogen was at all stages of growth much lower in respiration than plants abundantly supplied.

There are many complicating factors in studies of photosynthesis and it would be premature to draw conclusions, as Hamner points out. Nevertheless, it is of great interest that under conditions of light, nutrition and carbon dioxide supply that were apparently fairly satisfactory for wheat, his relatively dark green nitrate-supplied plants at first markedly increased and then later actually decreased in photosynthetic rate per unit of leaf area. Gregory and Richards (70) report for barley a similar response that was thought to be associated with advanced age of the plants. Hamner's low-nitrogen plants, high in carbohydrates and light green in color, exhibited during the same period of time no such decrease; they continued to increase in dry weight, the greatest gain in volume and dry weight being of the root system rather than of the tops. It

appears, according to Lundegardh (116), that the leaves of nitrogen-deficient oat plants were much more active photosynthetically than comparable plants which were supplied with nitrate.

Some of the various factors affecting the rate of nitrate assimilation will be considered later but it is clear that under conditions which favor rapid nitrate reduction and amino acid synthesis there may also be expected rapid utilization of carbohydrates or their derivatives, often resulting in very low carbohydrate reserves in the plant (57, 105, 153, etc., 215).

The carbon skeleton of the protein or amino acid molecule is necessarily derived directly or indirectly from carbohydrates. About 85 per cent of the more complex protein bodies are of carbohydrate origin although the nitrogen content of proteins is commonly emphasized. In addition to this, the fact of greatly increased respiration under conditions of nitrate reduction and amino acid synthesis explains, at least in part, why excessively heavy applications of nitrogenous fertilizers have frequently resulted in plants high in organic nitrogen but deficient in carbohydrates. Just what phase or phases of nitrate assimilation are most intimately associated with increased respiration is not apparent. Nevertheless, there is considerable evidence showing that an increase in the amino acid content of plants is associated with increased respiration (23, 232). Spoehr and McGee's (241) results are especially pertinent. They worked first with entire sunflower plants which were placed in darkness for 71 hours. During this period there was decrease in carbohydrates and, as usual, a material increase in concentration of amino acids undoubtedly owing mainly to proteolysis. Associated with the increase in amino acid content of their plants there was an enormous increase in respiration rate until the carbohydrate reserves became greatly depleted. Many of their experiments with detached leaves in darkness included the addition of amino acids to the water in which the cut ends of the petioles were immersed. For example, when glycine was supplied there was a gain in amino acid content and the leaves respired and decreased in carbohydrates much faster than other leaves which lacked glycine.

Discussion as to interrelations between these results and the increase in respiration that occurred following nitrate reduction would be premature. Still, the increase in respiration was undoubtedly associated with the appearance of amino acids, whether

supplied externally, formed through protein cleavage, or formed through new synthesis from reduction of nitrate and the oxidation of carbohydrates or their derivatives. Further, the comparatively high plane of respiration was maintained in tomato after nitrite could no longer be detected following the first flush of nitrate reduction (73).

Nitrate Reduction in Roots: The extracts of the fresh tissues of various plant organs have frequently been employed to measure the nitrate reducing ability of the structure concerned. Work of this type has been especially valuable in studies of roots (55, 59, 60, 155, 163, 259). Briefly, the technique for determining nitrate reductase activity, as developed by Eckerson (57, 58), consists of taking an aqueous extract of fresh plant tissue and measuring the amount of nitrite reduced from nitrate, in the presence of nitrate and glucose and some free oxygen, by a given sample under specific conditions of time, pH and temperature. It is, of course, essential to maintain a constant pH as well as a constant temperature, to avoid a deficiency of nitrate, glucose or oxygen and to eliminate microorganisms through the use of toluene. The amount of nitrite formed from nitrate gives a measure of the reductase activity of the particular plant or organ sampled. Analyses have repeatedly shown (57, 60, 155, etc., 259, 260, 262) that reductase activity closely parallels the synthesis in the plant of amino acids and other forms of elaborated nitrogen. Reductase activity, therefore, furnishes an index of the relative rate of nitrate assimilation.*

Although in the older literature (259) occasional statements may be found indicating that nitrate can be reduced to nitrite in the roots of plants, Thomas (259) seems to have been among the first to appreciate the significance of roots in nitrate assimilation. He conducted quantitative studies of the nitrogenous constituents of

* Erratic results in the attempted use of this method have recently been reported by Sommer (240), probably owing, in the opinion of the writer, to the fact that although she adjusted the initial pH of her plant extracts the pH was not controlled during the period of incubation (private communication). As recently emphasized by Hibbard (76), it is essential to bring the pH of the plant extract to pH 7.2 to 7.4, as recommended by Eckerson (58), and to so maintain it for the entire incubation period. Some plant extracts contain natural buffers which are adequate for this purpose; to others a phosphate buffer must be added. Dittrich (55) favors a pH value of 7.6 for nitrate reduction and it may be pertinent to mention, with further discussion later, that Dikussar (54), who grew maize with nitrite as the sole external source of nitrogen, employed a pH value of 7 in his culture medium.

apple trees through a year's cycle of growth. The material included mature and seedling trees receiving heavy applications of sodium nitrate at regular intervals throughout the vegetative period. In the aerial organs positive tests were obtained for nitrate, or nitrite, in one structure only and this at just one period of the year, in the leaf buds just as they were opening. On the other hand, the fine roots gave nitrate reactions throughout the season, although there was little in the main roots. It is highly significant that quantitative tests for amino acids were always higher in the roots than in the aerial parts.

Eckerson (59) has followed the reductase activity of apple trees during a year's cycle of growth and, in complete harmony with Thomas' chemical analyses, found that high reductase during the fall and winter was localized in the fine roots. The maximum reductase in early spring was localized in both fine roots and buds. There was very little reductase in the leaves at any time. Later work by Thomas (257, 258) is in harmony with his earlier observations and he further determined that, accompanying assimilation of nitrate, there was a marked increase in utilization of starch (260, 261). As already indicated, this would be anticipated since reduction of nitrate and synthesis of amino acids in roots can obviously not occur without oxidation of carbohydrates or their derivatives. Other work with apple trees, including many carbohydrate analyses, has corroborated the results of Thomas and Eckerson (159, 160, 163, 264, 265).

Stuart (245) reports that if nitrate is applied in extremely high amounts to small apple trees it may appear in the aerial organs. Accompanying the appearance of nitrate in the leaves, his trees exhibited severe scorching of the foliage; there was, however, no indication that this was directly caused by the presence of nitrate. His results do not minimize the value of the observations discussed above but furnish additional information. There can no longer be any doubt that in this species the reduction of nitrate and synthesis of amino acids takes place mainly in the roots.

In the asparagus plant Nightingale and Schermerhorn (155) found that active reduction of nitrate, both in darkness and in light, took place mainly in the fine rootlets. When the plant was in a condition of active vegetative growth of tops, nitrate was found only in the fine roots. In plants lacking nitrate in their tissues and

nutrient medium but containing reserve carbohydrates in their rhizomes and roots, an external supply of nitrate resulted in the appearance of nitrate, nitrite and ammonium in the fine rootlets only and not in the storage roots. At the same time, amino acids and asparagine appeared in these organs in considerable quantities and the amount of reserve carbohydrates was reduced. The results were later reflected in all parts of the plant. Computations on an absolute amount as well as on a percentage basis showed a striking decrease in carbohydrates and increase in organic nitrogen, as compared to plants that lacked nitrate.

The storage roots and actively growing tops of the asparagus plant apparently assimilated nitrate, but seemingly seldom had an opportunity to do so because nitrate was reduced in the fine rootlets before reaching other organs of the plant. If, however, the temperature was 10° C. or lower, nitrate was translocated to other parts of the plant, owing apparently to cessation of reduction at that temperature. Later with a rise in temperature, nitrate was assimilated by and disappeared from both the storage roots and the actively growing tops and was then found again only in the fibrous roots. In harmony with the preceding results, Eckerson (60) found that the tops or succulent shoots of young vigorously growing asparagus plants contained only traces of reductase, the new storage roots contained very little, and the older storage roots practically none, whereas the fine rootlets were in all cases high in reductase.

A similar transformation of nitrate to organic nitrogen occurred in the rootlets of narcissus bulbs both with plants continually in darkness and others under seasonal light. Reduction of nitrate resulted in a definite increase in organic forms of nitrogen and a decrease in carbohydrates as computed on a percentage and absolute amount basis (153). These results were in contrast to control plants that received no external supply of nitrogen.

Some underground storage organs, however, may reduce nitrate as, for instance, the roots of mangold (49). Dittrich (55) also found that the storage roots of plants of the family Chenopodiaceae reduced nitrate and he reported nitrate reduction by the roots of plants of several different families including the Graminaceae. Many other references might be cited but it will be sufficient to point out that in the Graminaceae Sani (217) found very active nitrate reduction by extracts of roots. It is notable that he found

the rate of nitrate reduction was doubled by extracts of maize roots when citric acid was introduced. Apparently organic acids or sugars may be oxidized in nitrate reduction. The assimilation of nitrate by the roots of the Graminaceae is in apparent accord with Hamner's (273) work (already discussed), wherein he reports a greatly increased respiration rate of the roots of wheat plants that received nitrate as compared to others lacking an external supply of nitrogen.

The importance of the roots of some plants in assimilation of inorganic nitrogen has been emphasized but the fact should be kept in mind that in many plants, such as tomato (57, 158), peas (164), soy beans, etc. (152, 60), the tops, rather than the roots, play the dominant rôle in reduction of nitrate and synthesis of amino acids. Before taking up in detail the matter of ammonium and nitrate nutrition it is of interest to note that Dittrich (55) found that beets when supplied with ammonium sulphate gave a negative test for reductase. Tiedjens (264) reports a similar situation for the extracts of apple roots when the trees in sand culture were supplied with the same salt. Nevertheless, he found, as did Davidson and Shive for peach trees (48), that both nitrate and ammonium assimilation took place mainly in the fine rootlets.

EXTERNAL FACTORS INFLUENCING AMMONIUM AND NITRATE NUTRITION

If vigorously growing plants in a complete nutrient medium are supplied with ammonium sulphate, the culture solution usually tends to increase in acidity, owing to the fact that the anions are absorbed by the plant in a smaller proportion than the cations. On the other hand, calcium nitrate under similar circumstances commonly causes an increase in alkalinity as the nitrate anion is usually absorbed with relative rapidity. Therefore, a mixture of ammonium sulphate and calcium nitrate in proper proportions for the internal and external conditions concerned, or under some circumstances the use of ammonium nitrate, may minimize pH changes of the residual nutrient solutions (267, 268, 303, 234, 191, etc., 130, 131, 63, 174, 176, 178). Because of the residual effect on the nutrient substrate, salts such as ammonium sulphate are commonly called "physiologically acid" and those such as calcium nitrate "physiologically alkaline." On the same basis ammonium nitrate is "physiologically amphoteric."

Nitrate Absorption: In the case of nitrate salts (as calcium nitrate), the differential intake of the two elements is probably wholly an expression of the ionic absorption (167). A calcium nitrate solution sufficiently acid to permit seemingly significant formation of molecular nitric acid (HNO_3) would probably kill the root hairs or other absorbing cells of most plants. Many investigations, some of which will be considered presently, show that a rather acid medium is more favorable for absorption and assimilation of nitrate than a neutral or slightly acid culture solution. Hoagland and Davis (78), working with the large-celled alga *Nitella*, found that penetration of nitrate was much more rapid from a slightly acid solution than from an alkaline one. But, directly or indirectly, it is probably assimilation rather than absorption of nitrate which limits growth. This is indicated by the responses of peach trees maintained by Davidson and Shive (47, 48) in sand culture with nitrogen supplied only as calcium nitrate at pH 6 (plus or minus .5). The trees removed relatively less nitrate from the nutrient medium and made somewhat less growth than comparable cultures at pH 4 (plus or minus .5) but lack of a nitrate supply within the trees of the less acid cultures was not a limiting factor. On the contrary, the peach tree, which ordinarily contains and assimilates nitrate mainly in the fine rootlets, had in this case nitrate not only in these organs but in the tops of the trees as well. Nitrate in the cultures at pH 4 was limited strictly to the roots (cf. *Nitrate Reduction in Roots*).

Their results and others to be discussed show that plants may absorb, assimilate nitrate and grow luxuriantly over a much greater pH range of the nutrient solution than is possible with the use of ammonium salts in solutions lacking nitrate. This would seem to indicate, along with accompanying records of differential absorption or shift in pH of solution, that adequate ionic dissociation of the nitrate salts occurs at the several pH levels concerned. Even at low temperature there seems to be sufficient dissociation for unhindered absorption of nitrate, as emphasized by Mevius and Engle (131) and frequently corroborated by the writer, the limiting factor with low temperature being assimilation rather than absorption of nitrate (155, 158, 160, 161, 163).

Ammonium Absorption: Mevius and Engle (63, 130, 131) also conducted extensive investigations designed to determine conditions

governing the absorption of nitrogen from solutions of ammonium salts at different pH values. The hydrogen ion concentration of nutrient solutions, one of the most critical environmental factors, was studied by observations of the responses of corn plants at different seasons of the year when supplied with ammonium sulphate in a complete nutrient solution at various concentrations and pH values. They conclude that when carbohydrate reserves in the plant are not a limiting factor, the effect of ammonium sulphate is determined in large part by the pH of the nutrient medium. This in turn determines the amount of cleavage of ammonium sulphate to free or molecular ammonia (NH_3 , or NH_4OH). They believe that the amount of free ammonia formed determines the amount of nitrogen which is available for absorption from a nutrient solution containing nitrogen only as the ammonium salt of a strong acid.

It is known that free ammonia is toxic to plants. This has been demonstrated by Willis and his collaborators (292, 293, 294). Their results indicate that where ammonium hydroxide has been successfully employed in significant concentrations as a source of nitrogen, its introduction into a nutrient solution or soil is associated with interaction of materials of the soil or nutrient substrate to form relatively insoluble compounds or non-toxic salts.

Mevius and Engle (63, 130, 131) record in support of their ideas the fact that the more alkaline the nutrient solution was the more rapid the absorption of nitrogen from ammonium salts. They further report that under the same conditions with high concentrations of the solution there was an increase in amount of absorption of free ammonia and death of the root tips. The surrounding residual nutrient solution increased in acidity owing to the usual differential absorption. Nevertheless, the death of the root tissues was preceded by an increase in alkalinity of the cells concerned that was apparently correlated with the presence within the cells of free ammonia. At pH values slightly below 6, large concentrations of ammonium sulphate were supplied without accumulation of free ammonia within the cells and without injury to the roots. Low temperature was also said to permit the addition of high levels of ammonium sulphate to the cultures without injury to the roots. The explanation given was that both low temperature and low pH limited the hydrolytic cleavage of ammonium sulphate to free

ammonia and, according to their hypothesis, must necessarily, therefore, have limited absorption of the nitrogen of this compound. On the other hand, Prianischnikov (196) claims that although nitrate absorption increased with rise in temperature that of the nitrogen of ammonium remained unaffected. Undoubtedly, the matter of assimilation was a dominant factor indirectly controlling absorption but the point to be emphasized here is that Prianischnikov, in accord with others, records absorption of nitrogen from ammonium sulphate under conditions that apparently eliminated the possibility of the presence of free ammonia in the culture medium.

In brief, Mevius and Engle (63, 130, 131) place the emphasis upon the absorption of molecular ammonia (NH_3 or NH_4OH) rather than upon absorption of the ammonium ion (NH_4^+), the product of electrolytic dissociation. They ascribe any injurious effects of "physiologically acid" ammonium salts to the accumulation of free ammonia in the plant cells rather than to the direct or indirect effect of residual acidity that may develop in the nutrient medium or at the absorbing surfaces of the roots. Their plant responses to the nutrient conditions employed are in excellent agreement with the work of many others if due allowance is made for the cultural technique followed by the various workers and the resulting degree of control of the pH of the respective nutrient media. This will be further discussed.

Unquestionably, Mevius and Engle have experimental evidence that supports their contentions but they probably have unduly minimized the importance of electrolytic dissociation of ammonium salts and the direct or indirect effects of residual acidity (*cf. The pH of the Nutrient Solution*). When the pH of the nutrient medium is nearly neutral or slightly alkaline, molecular ammonia may perhaps play the dominant rôle but there are many experiments indicating that ionic ammonium (NH_4^+) may be removed by plants from a nutrient solution at a pH value much too low for the hydrolytic cleavage from ammonium sulphate to ammonia (NH_3 or NH_4OH) (*cf. The pH of the Nutrient Solution*).

It is true with usual cultural conditions, as will be shown presently, that the absorption of the nitrogen of ammonium when coupled with assimilation is much more rapid at neutral or slightly acid pH than under more acid conditions of the nutrient medium. But it does not necessarily follow that *absorption* of the nitrogen of ammonium is necessarily a limiting factor in an acid nutrient solution.

Shive and his students (3, 38, 47, 233, 242), by analyses of residual nutrient solutions, have repeatedly determined that, at least under some circumstances the nitrogen of ammonium salts may be absorbed in measurable quantity by several different kinds of plants even though the pH values of the nutrient solutions were well below neutral. Very recently, Davidson and Shive (47, 48) grew peach trees in sand culture with excellent control of the pH of the nutrient solution. The entire external nitrogen supply for the trees under consideration was ammonium sulphate. The nutrient solutions were applied at pH 4 for some of the trees and the remainder received the same solution adjusted to pH 6 (plus or minus .5 in both cases). Absorption tests were conducted to determine the rates in milligrams per gram of dry plant material per hour at which nitrogen was absorbed by plants in the two treatments. These figures for the more acid series averaged for the growing season .052 as compared to .114 at pH 6. Further, the tendency of the culture solutions was to increase in acidity at both low and high pH values and, at least in the more acid culture, there could have been no free ammonia. The shift in pH must, therefore, have been correlated with differential ionic absorption of ammonium (NH_4^+). The trees at pH 4 exhibited a typical response in that they made less growth than those supplied with the less acid solution but the limiting factor was clearly not absorption for the percentage concentration of ammonium nitrogen (plus glutamine?) in the fresh absorbing roots was .011 and .013, respectively. The roots were not noticeably injured in either case (1932 series) although the volume of growth was greatest in the solution at pH 6. It is notable that Davidson and Shive say that the rootlets were relatively short in the pH 4 series. This is not a characteristic of trees deficient in organic nitrogen. Mevius and Engle, on the other hand, state that where the acidity of the medium was not low enough to cause root injury, plants at low pH supplied with nitrogen only as ammonium sulphate exhibited the same long slender type of roots that were characteristic of their cultures lacking nitrogen. Indubitably, this response is typical of nitrogen deficiency (*cf. Growth in Relation to Available Nitrate*) and supports their contention that at low pH values of the nutrient medium the nitrogen from ammonium sulphate was largely unavailable to the corn plant under the conditions of their experiments, but it is apparent that this does not hold true.

for all plants under some environmental conditions. As will be shown presently, internal factors and other external factors in addition to pH, very greatly modify the utilization of ammonium nutrients, as for example, the relative amounts of calcium and other non-nitrogenous ions. It may also be mentioned, with further discussion in the following pages, that some plants in an acid medium may absorb ammonium without there occurring any noticeable root injury, whereas others not only fail to absorb but actually excrete ammonia, thereby tending to neutralize external acidity. The difference in plant response to the acid environment of the roots seems to be determined by the quantity and proportions of protein and carbohydrate reserves (188, 191, 192).

The same general principles that have been discussed apply to ammonium nitrate. However, consideration to it can be most conveniently given elsewhere as the responses of a plant to nutrition with this "physiologically amphoteric salt" are closely correlated with metabolic activities. Likewise a discussion of Prianischnikov's views and evidence concerning the effects on plant cells of residual nutrient acidity and free ammonia can be presented to better advantage a little later.

The pH of the Nutrient Solution: The importance of the hydrogen ion concentration of the nutrient solution has been emphasized but a catalogued list of the experimental results of various workers recording the pH which gave best growth for each particular set of experimental conditions would avail little. It must already be apparent to the reader that there is no one best pH value for a given nutrient solution for all plants nor for the same kind of plant under different environmental conditions. Mevius and Engle (63, 130, 131), whose work has already been discussed, found that ammonium salts could be supplied to corn plants without injury during the summer months at a pH value considerably less acid than could be employed in the winter months when light conditions were less favorable for carbohydrate synthesis. Although they strongly emphasize the matter of pH of the nutrient solution they are not less emphatic in pointing out the importance of other factors. In a general way, under excellent control of pH, it may be said that at pH 5.3 to 5.6 they obtained approximately equal growth with both nitrate and ammonium salts.

When nitrate was applied at much less acid pH, corn was detrimentally affected, apparently owing to iron deficiency. This ele-

ment is notably difficult to keep in solution as the culture medium shifts towards alkalinity (210) when there are present salts such as calcium nitrate. As will be shown presently, this is apparently owing in part to differential absorption and the resulting increase in alkalinity at the surface of the absorbing root where it causes iron precipitation. This may occur even though the bulk of the nutrient solution is sufficiently acid to maintain iron in solution (159). Internal factors may, however, be of equal or greater importance (210). Pirschle (177, 178) worked with different plants and noted that, while certain kinds made excellent growth with nitrate supplied at an initial pH well above the neutral point, several species were limited in growth at least in part by inability to absorb iron under these conditions. In general, he found, as many others have, that nitrate gave excellent growth responses over a much greater pH range than ammonium. Nitrate utilization and growth was distinctly favored, however, by a pH value in the vicinity of 5, varying somewhat with other external and internal conditions to be discussed. In the case of some plants he obtained two pH optima; although the reasons for this are not entirely clear in every case, he indicates that in general it was associated with indirect effects of the hydrogen ion concentration resulting in the harmful effect of iron deficiency or in interference with the absorption of other non-nitrogenous ions. He states very pertinently that there is no absolute pH optimum and that his statements about it are only approximate for the immediate conditions of his experiments. With these reservations he concluded that under a system of constant renewal of nutrient solutions most plants had their optima between pH 5.5 to 6.5. Within this range there was usually very little difference in the growth responses of ammonium- and nitrate-supplied plants. Loo's (112) extensive experiments are in harmony with those just cited. He reports different pH optima but, in general, nitrate was most available at a weakly acid reaction and ammonium at a neutral or slightly alkaline pH value.

The work of Shive and his students and of Prianischnikov and his collaborators carried on over a number of years is in complete accord with the preceding observations but as their work is of additional significance in its bearing upon metabolic responses it will be discussed later in that connection along with the results of Tiedjens and others. It should be mentioned here, however, that in earlier

experiments Tiedjens and Robbins (263) reported that tomato plants supplied with ammonium nitrogen at an initial pH of 8 absorbed nitrogen and grew luxuriantly with uninjured roots. This observation, in apparent contrast to others already cited, is not actually in conflict in principle for the initial pH was subject to rapid change in their sand culture medium, especially as the root systems increased in volume.

It may well be asked what the real pH of a nutrient solution is, if subject to shift in value as a result of more or less continual differential absorption of anions and cations. The answer must be that only a range of pH values can be determined. Apple trees supplied with a complete nutrient solution were employed in a test involving the determination of the pH ranges occurring in cultures supplied with ammonium sulphate at pH 6 or with calcium nitrate at pH 4.5 (159). The pH values were not chosen arbitrarily but were those found by Tiedjens (265) to be favorable for the growth of apple trees in sand culture under conditions of constant solution renewal. The trees were grown at a practically constant temperature of 10° C. in both sand and water cultures. In both media the solutions were constantly renewed according to the method of Shive and Stahl (234) at the rate of 36 liters per culture every 24 hours. This rate of renewal was such that the solution after bathing the roots and passing out of the culture vessel did not change more than plus or minus .1 pH. As usual, the ammonium sulphate cultures tended to become more acid and the calcium nitrate cultures more alkaline. Absorption and assimilation of both ammonium and nitrate was verified by plant analysis. The results were very definite as there were available for comparison similarly treated trees that were grown with no external nitrogen supply at pH 5.

Associated with absorption of nitrate and ammonium, respectively, and with nearly perfect control of the pH of the bulk of the solution, it was found by the range indicator method of Small (236) that the trees in sand culture receiving ammonium sulphate at pH 6 had, at the absorbing surface and tips of the fine fibrous roots, a pH of 4 to 4.5. The sand immediately adjacent to the roots also had about the same H-ion concentration, with a gradient reaching pH 6 at about 2 cm. from the absorbing surface of the root. The calcium nitrate cultures at pH 4.5 had, at the absorbing surface and tips of the fine roots, a pH of 5.6, as did also the sand in contact with the

roots; although less than 1 cm. from the roots the solution on the sand particles was about pH 4.5. On the other hand, the minus-nitrogen cultures at pH 5 did not noticeably affect the H-ion concentration of the solution as a whole, and the surface of the fine roots appeared to have a pH value of approximately 4.8 to 5. Yet the roots, although somewhat more slender, were growing, at least in length, at about the same rate as in the case of the trees receiving nitrogen in the nutrient solution.

The ammonium and the nitrate water culture series were vigorously stirred with a motor-driven agitator. Even under these conditions the ammonium sulphate (pH 6) cultures had at the surface of the fine absorbing roots a capillary film of pH 5.4 while the trees supplied with calcium nitrate nutrient solution at pH 4.5 had at the surface of the absorbing roots a film of pH 5.2. It is obviously impossible, in sand culture, absolutely to control the pH of the solution bathing the roots, if the plant is absorbing from the nitrogen-containing salt of the nutrient solution in largest part ammonium from ammonium sulphate or nitrate from calcium nitrate. The preceding results would not indicate that a widely different pH of the nutrient medium is required for ammonium from that required for nitrate nutrition. Actually, the trees of the ammonium series assimilated nitrogen rapidly when the solution bathing the roots was pH 4.5, and those of the nitrate series when the absorbing surface of the roots was pH 5.6. Practically, under conditions of sand culture it is essential that the initial solution containing ammonium sulphate be approximately neutral (pH 6) and that of a calcium nitrate culture be relatively more acid, in order that the absorbing root surfaces may not become extremely acid in the former case or excessively alkaline in the latter case.

Non-Nitrogenous Ions: Prianischnikov and his students over a considerable period of years have been studying in part the effects of the pH of the nutrient medium on ammonium and nitrate nutrition (191, 194, 196, 53, 54, 85, 86). Their results are very similar to those already cited. In addition, they determined the ash content of a considerable number of species of plants and found almost without exception that their ammonium-supplied plants contained much less calcium than similar series receiving nitrate (.18 against .32 per cent CaO in sugar beet). Upon the addition of more calcium to the nutrient solution there was greater intake of this element and

good growth of sugar beet was obtained when ammonium sulphate was supplied at a practically constant pH of 4 (constant renewal culture). The recorded yield in this particular instance was slightly greater than that of comparable cultures with the same or less calcium at pH 6 (194). Other tests were conducted in which the pH was controlled by either flowing cultures or frequent additions of increments of acid or alkali as required to solutions containing ammonium sulphate. The results were similar to those described. Diminishing the amount of calcium was unfavorable to the growth of the plants in the ammonium cultures. Potassium was much less marked in effect although an abundance seemed more essential in ammonium than in nitrate nutrition. High concentrations of calcium were distinctly detrimental to plants of the nitrate-supplied series at both high and low pH values of the culture solutions.

Holly *et al.* (80, 81, 82), working with ammonium and nitrate-supplied cotton plants in sand culture, report that the use of the ammonium ion as a source of nitrogen reduced the absorption of bases, the greatest effect being on calcium and magnesium. Although the use of ammonium was associated with reduced calcium absorption there was no evidence that the presence of calcium was correlated with a reduced absorption of ammonium. They state in addition that the differences in calcium content between the nitrate and ammonium-supplied plants was due principally to leaf calcium content. The differences in magnesium were evident, however, in roots, stems and leaves. Differences in absorption of sulphate and phosphate were small and varied at different stages of development.

Ivanova (85), likewise using cotton, reports that in sand culture best growth was obtained at pH 7 but that through the introduction of additional calcium to Naftel's (147) solution as calcium chloride or sulphate, cotton was able to utilize ammonium at pH 3 throughout the vegetative period. Additions of potassium had no apparent effect and increased magnesium as the sulphate or chloride was detrimental. In comparable studies with sugar beets, cabbage and flax, Dikussar (53) found that ammonium at pH 7 gave as good yields as nitrate at pH 5 and he obtained responses similar to those described through the use of different proportions of calcium and magnesium. Various other ions have been investigated (45, 46, 177, 178, 281) to determine their relationship to ammonium and nitrate utilization but the results permit no conclusions, probably

partly because of precipitation of materials in the nutrient solution at the more alkaline ranges or of variations in experimental conditions.

Considerable additional work on calcium has been reported by Prianischnikov and will be considered later in connection with metabolic responses (189, 196). It is significant that he strongly emphasizes the fact that there is no single coefficient through which the optimum pH value of a nutrient medium can be determined. It depends not alone upon such external conditions as the concentration, temperature and calcium content of the nutrient solution, but upon internal factors presently to be discussed.

Stage of Plant Development: Shive and his students have made extensive quantitative studies of the absorption of nitrogen by oat and buckwheat plants which received equal proportions of nitrogen as ammonium and as nitrate at various stages in the life cycle of the plants (233, 242). They found that the rate of absorption of nitrogen as nitrate by oats was lowest in early growth, reached a maximum at the blossom stage and then declined. Ammonium, on the other hand, was absorbed most rapidly during early stages and declined with increasing age of the plant. The absorption of total nitrogen also reached a maximum at the flowering stage. Quantitative determinations of the nitrogenous materials of the oat plants served further to verify the conclusions arrived at through analysis of the residual nutrient solutions. With buckwheat, on the contrary, ammonium absorption predominated over nitrate absorption during the greater part of the life cycle of the plant. The nitrate absorption rate exceeded that of ammonium only in the very late stages of growth when the rate of intake of both forms of nitrogen was extremely low.

Later work with tomato in which ammonium and nitrate nitrogen was supplied in equal proportions, indicated that ammonium absorption tended to predominate over that of nitrate in young plants, but the pH value of the nutrient media produced a very striking response (3, 37, 38). This was decisively demonstrated by shifting plants from solutions of one pH to those of another pH. Twelve days later the plants were tested as to their rates of absorption. The effect was to retard the rate of nitrate intake regardless of whether the transfers were made from solutions of relatively high to those of low pH values, or *vice versa*. On the other hand, plants transferred

from pH 7 solutions to cultures at pH 4 or *vice versa* for immediate absorption tests showed that the effects on the rates of absorption of ammonia were immediate. Although the reaction change exerted an immediate influence upon nitrate intake the effect was not so pronounced as in case of ammonium absorption, possibly because of the high nitrate content in the plants of the several series. Ammonium, in contrast, was present as usual only in small concentrations.

The results with oats are in harmony with the responses of young cotton plants as observed by Naftel (147). This species first absorbed more ammonium but later more nitrate. Prianischnikov (197) repeated Naftel's experiments and came to the conclusion that the character of the culture solution employed by Naftel, rather than the stages of development of the plant, was the determining factor. In Prianischnikov's opinion the plants had received a great excess of nitrogen (80 mg. N per L. every two days). He considers that the comparatively high proportion of magnesium sulphate employed must on account of the abundant sulphate have favored the absorption of nitrate (*cf.* Loo, 112) which in conjunction with less calcium than magnesium indicated to him a solution particularly adapted to nitrate rather than ammonium nutrition.

Prianischnikov, in repeating Naftel's experiments, took as a starting point his solution "C" which contained equivalent amounts of ammonium and nitrate. The unmodified solution gave results similar to those of Naftel but with a doubled amount of calcium introduced as the chloride or sulphate, the plants absorbed in every stage of development more ammonium than nitrate and made more growth. The following data indicate the results obtained:

Age of plants in days		Mg. nitrogen absorbed per 24 hours per culture				Dry wt. of plants per culture grams
		30	60	80	100	
Nutrient solution "C" of Naftel at pH 4.8	NH ₄ -N NO ₃ -N	9.3 15.6	8.7 11.9	12.2 13.4	6.9 6.0	30.7
Same solution "C" with double the calcium, pH*	NH ₄ -N NO ₃ -N	19.9 9.8	37.5 19.4	29.6 23.6	19.8 11.3	67.1
Same solution "C" at pH 7	NH ₄ -N NO ₃ -N	13.8 7.1	20.6 17.9	24.0 15.1	10.8 6.8	58.6

* Presumably pH 4.8.

Prianischnikov concluded, therefore, that the pH and calcium content of the solution exerted more influence upon the relative absorption of ammonium and nitrate than the stage of plant development.

He suggests that the results obtained by Stahl and Shive (242) with oats were associated with the fact that they employed a nutrient mixture high in nitrogen (240 mg. per liter) and supplied it as a flowing culture. [This does not, however, account for the responses with buckwheat nor for the fact that equivalent amounts, or greater, of ammonium or nitrate supplied separately will carry tomato plants through their entire life cycle (37, 38, 39, 265).] When Prianischnikov furnished oat plants with the same solution containing only 24 milligrams of nitrogen per liter, he recorded in all developmental stages greater absorption of ammonium than of nitrate. His results agreed with those of Stahl and Shive when the same amount of nitrogen was used, *i.e.*, in later stages of growth there occurred mainly nitrate rather than ammonium absorption. He obtained the greatest dry weight yield of plant material when the nitrogen supplied was 48 mg. per liter. (*cf. GROWTH IN RELATION TO AVAILABLE NITRATE*). His plants began to excrete ammonia into the external medium after 20 to 30 days when receiving 480 mg. per liter of nitrogen; at least, his solutions gained in amount of ammonium and became less acid whereas nitrate in the solution decreased. He ascribes the excretion of ammonia to continued absorption and reduction of nitrate by the plants without further assimilation to organic nitrogen.

These results are not of minor importance but, as will be indicated presently, Prianischnikov's work and that of many others emphasizes the significance in nitrogen nutrition of the chemical constitution of the plant. Detailed descriptions and carbohydrate analyses of the plants of the several experiments cited would have been of value. Obviously, the change in appearance of plants with different stages of development is an external expression of a continually changing internal status. The pH and calcium content of the nutrient solution and other external factors are clearly important but not to the exclusion of the internal condition of the plant which is often the chief limiting factor in nitrogen nutrition. A discussion of such relationships follows.

INTERNAL FACTORS INFLUENCING AMMONIUM AND NITRATE
NUTRITION

The pH of Root Cells: Conrad (42) grew maize seedlings in a complete nutrient solution in which nitrogen was supplied as ammonium nitrate. After some growth had been made the plants were shifted to single salt solutions of ammonium sulphate and potassium nitrate as well as to solutions containing, respectively, only the corresponding acid. The initial pH of the salt-containing cultures was about 5.8; that of the acid-containing media, approximately 2.4. The length of time the plants remained in these cultures is not stated but it was apparently for a sufficient length of time to affect more or less complete removal of nitrogen from the solutions containing it. At least the ammonium sulphate cultures showed a shift in pH value to 2.7 and the potassium nitrate and nitric acid series to 6.5 and 6, respectively. There was a decrease of .2 only in pH value of the sulphuric acid solution. Comparable cultures of sodium nitrate and carbonate gave residual pH values of about 7.2.

It is not made clear whether the pH values of cultures ranging from 2.7 to 7.2 in any way affected the external or internal appearance of the roots, but the composite tissues of roots, stems and leaves from single cultures were dried in an oven at 70° and ground. Aqueous suspensions of this material from the several series gave pH values ranging from 5.1 to 5.7 which corresponded, respectively, to nutrient media with the residual values of 2.7 and 7.2.

Keyssner (92), using flowing complete nutrient solutions maintained at practically constant pH, supplied oat plants and various other species with combinations of ammonium and nitrate to which had been added dilute acid or alkali as required. The pH values of his solutions ranged from 4 to 9. The plants were grown in the respective solutions for about two weeks, after which the roots were washed in distilled water and ground to a pulp. The pH of this freshly ground pulp was determined with the quinhydrone electrode. In typical experiments the root sap of nitrate-supplied cultures varied from 5.6 to 8 and that of corresponding ammonium series from 6.7 to 7.7; the external pH of the cultures, going in the same direction, ranged from 4 to 9. It is significant that in cases where the extreme pH values of root sap occurred the plants were

badly stunted. For example, the dry weight yield of plant substance for the ammonium series was 38 grams when the culture pH was 9 as compared to a maximum yield of 481 grams at a nutrient solution pH of 6 and a sap pH of 6.3. Various other experiments might be described in which composite extracts of all parts of the plant or single organs consisting of various tissues have given one or another result (46, 236).

Such results are difficult of interpretation as in a single cross-section of a root tissues will usually be found having pH values ranging from less than 4 to about 7 (236). It is obvious that if the nutrient treatment is associated with change in relative proportions of the respective tissues there will be a change in pH of extracted sap, yet during the same period cells of a given tissue at a given stage of development may not have exhibited any change in hydrogen ion concentration. Juices expressed from complex plant tissues have a hydrogen ion concentration which represents the algebraic sum of that of the sap of phloem, xylem, parenchymatous tissue, any specialized cells present, dead tissue, etc.

Hoagland and Davis (78) have eliminated this objection by employing the expressed sap of single cells of *Nitella*. They found that the hydrogen ion concentration of healthy cells of this plant was approximately constant at pH 5.2. The nutrient media employed by them varied from pH 3.8 to 9.4, yet no appreciable change of pH occurred in the cell sap, except below 5, and at the lower values they state the cells were unquestionably injured. In connection with studies of penetration of ammonium and nitrate ions which have already been mentioned, they noted that the ammonium ion penetrated rapidly and caused a change of reaction in the cell sap. With .005 molecular ammonium salts, the reaction was, in most cases, changed from pH 5.2 to pH 5.6-6.2 in the course of 24 hours or less but the cells were injured.

Cells of a kind at a given stage of maturity do not apparently have an absolutely constant pH value. Young respiring cells seem to fluctuate more than mature elements (236, 163, 159), presumably owing at least in part to the relative rate of respiration and effects of carbon dioxide under different conditions (65). But aside from fluctuations within rather narrow limits, comparable cells of a given tissue probably maintain a fairly constant hydrogen ion concentration. Observations of the tissues of the roots of apple trees

grown at a practically constant temperature of 10° C. with ammonium and nitrate, respectively, at various pH values of the nutrient media were in accord with this view (159). Associated with a high degree of residual acidity in cultures supplied with a high concentration of ammonium sulphate, the cortical cells became abnormally acid but this was associated with severe injury. The roots were short, stubby and bulbous in appearance. This was due mainly to the development of the primordia of lateral roots, most of which never developed sufficiently to emerge through the cortex, probably because of the extremely acid condition of the root surface and of the outer cortical cells which were about pH 2.8 to 3. There was no evidence of increased alkalinity owing to the absorption of ammonium, which certainly occurred as indicated by the increased ammonium and organic nitrogen content of the root system as well as by the shift in pH from 6 to the values mentioned. This shift in pH occurred only at or near the root surfaces, not throughout the nutrient medium.

It should be emphasized, however, that these trees were grown at low temperature preventing thereby, according to Mevius and Engle (130, 131), the hydrolytic cleavage of ammonium sulphate to molecular ammonia. The trees also had an unusually high carbohydrate reserve, an important factor in detoxication of ammonia (see this heading). There would seem no question but that in this case injury to the roots was caused directly or indirectly by the residual acidity of the nutrient medium. This is not necessarily in conflict with the observations of Mevius and Engle, Ribbert and others (204), who have reported that although accompanied by residual acidity the major factor bringing about injury to plant tissues was probably the presence of free ammonia in the cells. In addition to tests with culture solutions, Ribbert injected leaf cells with ammonium sulphate and obtained an increase in alkalinity of the cells which he ascribes to the probable presence of free ammonia. It would seem obvious that, if extreme, either external acidity or internal alkalinity could well result in injury to plant tissues. It should be emphasized again that the gross pH value of the nutrient medium as a whole gives no measure of the hydrogen ion concentration at the absorbing surfaces of the root.

Regardless of the origin of the free ammonia, the degree of pH change of the protoplasm and the extent of injury from it will be

dependent very largely upon the capacity of the plant for disposal of any ammonia present in the tissues. Much of the following discussion is concerned with this matter.

Sources of Ammonia: (1) To recapitulate, one source of ammonia is that formed from the cleavage of proteins, polypeptides, etc. The resultant amino acids are deaminized, leaving the carbon skeleton of the original amino acid plus free ammonia. If carbohydrate reserves are adequate or there is opportunity for carbon dioxide assimilation, detoxication of ammonia takes place through synthesis of one or both of the amides, asparagine and glutamine, some free oxygen being required for amide synthesis, however, even though carbohydrates may be present.

(2) There is also the possibility, emphasized by Mevius and Engle, of direct absorption of free ammonia (NH_3 or NH_4OH) from a neutral or slightly acid nutrient solution containing ammonium sulphate or a similar salt. However, with absorption of the nitrogen of ammonium sulphate there is rapidly developed considerable residual acidity, especially at the absorbing surfaces of the roots. The resultant pH value is much too low for hydrolytic cleavage and formation of free ammonia. At least in a nutrient medium much below neutral, plants probably absorb the nitrogen of ammonium sulphate as the ammonium ion (NH_4^+) (167).

(3) The reduction of nitrate with accompanying oxidation of sugars or their derivatives may take place in darkness or in light, the first product to appear being nitrite and then ammonia. Regardless of the initial source of free ammonia, other conditions being equal, its subsequent disposal by the plant will be the same whether it was derived through protein cleavage, by direct absorption or through reduction of nitrate.

Disposal of Free Ammonia by the Plant: The preceding observations are clearly in harmony with Prianischnikov's now generally accepted theory that ammonia is the "alpha and omega" of nitrogen metabolism in the plant (188, etc.). Much of his work and that of Mothes (135), already discussed, has emphasized the fact that ammonia was not stored in the plant as such but was metabolized to amides in the presence of carbohydrates and thus rendered innocuous.

Storage as Ammonium Salts: In a series of papers published a few years ago, Ruhland and Wetzel (212, 213, 214, 291) have

shown that in plants with a very acid sap there may be storage of ammonia as the ammonium salts of organic acids, not free or molecular ammonia. For this there is required not only a low pH value but actual acidity as well. Some of their first observations were made with the leaves of *Begonia semperflorens* which had a pH value of about 1.5 and contained 20 per cent of their dry weight as oxalic acid. In a typical experiment some of these leaves were placed in darkness at 28–35° to bring about carbohydrate deficiency and proteolysis (cf. *Metabolism of Leaves*). At the end of 106 hours the ammonium nitrogen amounted to 30 per cent of the total nitrogen whereas the small amount of initially present amide completely disappeared. Accompanying protein cleavage and loss of amide nitrogen they record an increase in acidity to pH 1.3, which they believe was directly correlated with deamination. The metabolized acid, whatever the origin, was apparently adequate to react with the ammonia formed proteolytically so that no free ammonia injury was possible.

Ruhland and Wetzel worked also with the usual garden rhubarb, *Rheum hybridum* Hort, the rhizome of which had only a slightly acid reaction and contained about the usual distribution of amino and amide nitrogen and no more than traces of ammonium nitrogen. As the young leaves developed there was rapid growth and protein synthesis with accompanying decrease in the amide and amino nitrogen of the rhizome. As the leaves approached maturity there was apparently deamination to the extent that the petioles of fully developed leaves contained about 60 per cent of their total nitrogen as ammonium. Correlated with deamination—or at least it is so considered by Ruhland and Wetzel—there appeared first malic and succinic acids chiefly, followed, as the petiole matured, by an increase in oxalic acid and a decrease in the acids mentioned. There was present at all times an adequate amount of organic acid to prevent the appearance of injurious free ammonia.

Ruhland and Wetzel's interpretation of the organic acid metabolism of rhubarb has been criticized by Bennet-Clark and Woodruff (13) who have reinvestigated the matter, conducting their experiments in a manner to permit computations on an absolute amount basis. They concluded there is little justification for the view that malic acid is derived from the carbon residues of deaminated amino acids. They did not question the ability of the rhubarb petioles to

store ammonia as the ammonium salt of organic acids but emphasized that their results showed that the increase in organic acid content of the petioles is associated with translocation in the spring and that malic acid is apparently a product of carbohydrate metabolism.

Recent work by Kultzscher (106) and Rahn (200) gives further evidence to show that highly acid plants store ammonia as the ammonium salts of organic acids. Kultzscher, in addition, supplied acid plants, *Begonia semperflorens* pH 1.4 and *Oxalis deppei* pH 1.3, with calcium nitrate and found that there was a striking increase in ammonium stored as the salts of organic acids, presumably indicating nitrate reduction to nitrite and ammonia with delayed or limited assimilation of the ammonia. Other plants, the expressed sap of which gave a pH value of about 5, followed the conventional course of metabolism, amides being metabolized with increase in ammonia from nitrate reduction or deamination. They describe also plants which are intermediate between the so-called "ammonium plants" of Ruhland and Wetzel and the "amide plants," the type upon which all the earlier work was based. In general, the intermediate plants had an intermediate pH value of expressed sap but the ratio of amide to ammonium bore no constant relationship to the hydrogen ion concentration of the heterogeneous sap extract. It will be recalled that Wood (296), in his studies of *Atriplex nummularium* leaves, found marked variations in the proportions of ammonium plus amide and amino nitrogen depending upon the pH values of the tissue. Unfortunately, he did not determine ammonium and amide nitrogen separately.

Both Kultzscher and Rahn follow Ruhland and Wetzel's hypothesis and concluded, on the basis of percentage determinations, that the storage organs of acid plants typically are high in amino nitrogen and that with expansion of new shoots there is rapid deamination, the carbon skeleton of the amino acids concerned forming organic acids which react with the ammonia produced from the amino group to form ammonium salts. In the storage structures of the "amide plants" there was nearly as much amide as amino nitrogen.

Detoxication of Ammonia: The matter of detoxication of free ammonia has already been discussed in connection with formation of ammonia from proteinaceous reserves contained in various organs

of the plant. A few experiments will be cited sufficient to indicate the apparent course of metabolism in detoxication when the ammonia is derived from external sources. The investigations of Prianischnikov and his collaborators with seedlings in darkness and in light furnish excellent illustrations (183, 184, 185, 186, 187, 189, 190). Summing up briefly the work of many years of research, it may be said that seedlings such as oats, barley and maize, relatively high in carbohydrate reserves, and pumpkin, containing reserve fats, absorbed ammonium rapidly in darkness or in light from solutions containing ammonium sulphate or chloride. This was associated with no appreciable accumulation of ammonium but with a marked increase in asparagine or in glutamine, the latter amide occurring especially in the Cucurbitaceae. If seedlings containing abundant nitrogen-free reserves were grown in darkness until the carbohydrate or fat content became practically exhausted and were then supplied with ammonium, there was practically no elaboration of amide nitrogen; ammonium accumulated probably in part as free ammonia and the plants died. Others received an external supply of glucose, synthesized asparagine, accumulated little ammonium and were not injured.

Seedlings such as *Vicia sativa*, *Vicia faba* and *Pisum sativum* were unable to elaborate asparagine from absorbed ammonium except when there was present in the external medium an abundance of calcium, as the chloride, sulphate or carbonate. Work was done also with low-carbohydrate seedlings such as *Lupinus* that store mainly hemicelluloses. *Lupinus*, even with addition of calcium, was unable to synthesize asparagine from absorbed ammonium in darkness. The seedlings were accordingly injured. But it is notable that, with an external supply of glucose in darkness or when the seedlings were grown in light, formation of asparagine occurred, there was little increase in concentration of ammonium in the plants and they were uninjured. Burkhart (24) very recently studied the metabolism of etiolated seedlings, some of which received no external nitrogen supply and others a typical ammonium sulphate nutrient solution. He found an increase in absolute amount of organic nitrogen, owing to assimilation of ammonium in darkness, in case of all seedlings employed except *Lupinus*. In other seedlings containing abundant carbohydrate reserves, he obtained striking increases in organic nitrogen as a result of assimilation of am-

monium in darkness. He obtained the usual formation of asparagine during the early period; then, following or accompanying carbohydrate depletion, there was proteolysis and increase in ammonium. In cases where sugars became extremely low there was injury and presumably the formation of free ammonia.

Smirnov (237) studied in some detail the metabolism of etiolated seedlings of *Hordeum sativum*. During the early period in darkness there was an increase in amino acids owing to assimilation of ammonium derived from a nutrient solution containing ammonium chloride. As carbohydrates were consumed, amide nitrogen increased and with extreme depletion asparagine was no longer elaborated, ammonium or free ammonia accumulated and the plants were injured. With no attempt at explanation, it is of interest to note that calcium appeared to accelerate the several metabolic steps in both synthesis and hydrolysis of proteins. It was associated with more rapid decrease in dry matter.

Smirnov supplied some of his plants in darkness with ammonium malate and succinate, respectively, for comparison with ammonium sulphate cultures. The salts of these organic acids were associated with an apparently significant increase in synthesis of amino acids and asparagine but for further synthesis to proteins the presence of abundant glucose was required. In this connection it may be said that the technique of infiltration indicated synthesis of asparagine from ammonium malate. This synthesis recorded by Mothes (140) occurred in leaves which were deficient in carbohydrates (*cf.* 18, 19).

Many more examples might easily be given but sufficient evidence has been presented to show that detoxication of ammonia in plants of the "non-acid" class is often dependent in large part upon the ability of the plant to elaborate asparagine or glutamine. For this purpose the presence of reserve carbohydrates in darkness is essential, or sunlight and the opportunity for CO₂ assimilation. This varies with seasonal conditions and location and although seldom considered is very frequently a serious limiting factor in studies of nitrogen nutrition.

*Excretion of Ammonia**: The phenomenon of ammonia excretion was observed by Prianischnikov when pea seedlings were tested as to their response to a nutrient solution containing ammonium nitrate (188, 191, 192). It was noted that in comparatively acid solutions

* Ammonia and volatile amines have been reported in the exhalate of certain flowers (97).

there was excretion of ammonia by the roots instead of absorption which occurred in neutral or slightly acid media. Other seedlings were selected for trials so as to obtain plant material containing different proportions of protein and carbohydrate reserves. For example, in oats the proportion of protein to carbohydrates is about 1:6; in peas, 1:2. Results were obtained as follows:

	Concentration of HCl			
	0.00075 N		0.001 N	
	Oats	Peas	Oats	Peas
First occurrence of NH ₃ after ..	19 days	4 days	12 days	3 days
Death of the plants after	20 days	7 days	15 days	5 days
Initial pH of media.....	3.1	3.1	2.9	2.9
Final pH of media	5.1	5.4	5.1	5.6

The results are in accord with the previous discussion in showing that the appearance of ammonium and ammonia is dependent in part upon the ability of the plant to synthesize asparagine which in turn is dependent upon the presence of carbohydrates. They indicate, in addition, that with increase in acidity of the nutrient medium the appearance of ammonia was greatly accelerated and was accompanied by injury to the plant.

Following these and similar tests with seedlings a large number of trials were made employing ammonium nitrate and calcium or sodium nitrate as the nitrogen source. The object was to obtain further information concerning possible excretion of ammonia from plants high and low in carbohydrates when grown in culture solutions of different degrees of acidity (191, 193, 195, 196, 86, 115). Their results were consistent in showing that when plants were supplied with a nutrient solution containing ammonium nitrate, ammonium was invariably absorbed more rapidly than nitrate as long as the carbohydrate reserves in the plant were abundant and the pH of the nutrient media as a whole did not drop below pH 5. With decrease in carbohydrate reserves, whether it was an inherent condition of the plant or the result of etiolation, nitrate was absorbed rather than ammonium. However, regardless of how high the carbohydrate content, nitrate absorption predominated when the nutrient media *en masse* was less than pH 5. By greatly increasing the relative proportions of nitrate in an ammonium nitrate medium

through the addition of calcium nitrate, there was, even at neutral or slightly acid values of the solutions, some increase in nitrate utilization; but the dominant factors in determining the relative importance of the respective nitrogenous ions was, as indicated, the available carbohydrate supply and the pH of the nutrient solution.

When for any reason carbohydrates approached depletion in plants supplied with ammonium nitrate, then, as usual, the formation of asparagine or glutamine was inadequate for detoxication of ammonia. Eventually death occurred but before carbohydrates became depleted to the point of practical exhaustion of supply there occurred a striking increase in ammonia in the nutrient medium as already stated. This was greatly increased when the cultures were relatively acid.

Perhaps the most surprising phenomenon was the continued reduction of nitrate by plants that were rapidly decreasing in carbohydrate reserves. However, there was apparently no new synthesis of amino acids, the reaction stopping with formation of ammonia. This was most pronounced in acid media. Analyses of residual solutions of cultures supplied with ammonium nitrate, in which had been placed low-carbohydrate plants, showed continued decrease in nitrate in the nutrient medium but very definite increase in ammonium. Many tests with ammonium nitrate indicated this to be true. In addition, plants were supplied with a complete solution containing nitrogen only as nitrate and there occurred absorption of nitrate and increase in ammonia in the residual solution when carbohydrates in the plant were too low to permit formation of asparagine or glutamine with resultant detoxication of the ammonia originating from nitrate reduction.

There are in the literature the results of a considerable number of experiments involving the relative absorption rates of nitrate and ammonium from solutions containing these two ions in various proportions. The results of many of these determinations have already been cited in connection with absorption rates at different stages of development of the plant. It will be recalled that Prianischnikov (197) reported that mature plants (carbohydrate content unknown), when supplied with high concentrations of ammonium and nitrate, absorbed the latter but that there occurred, instead of synthesis of organic nitrogen, considerable storage of nitrate and reduction to ammonia which was excreted into the nutrient media.

COMPARATIVE METABOLISM OF AMMONIUM- AND NITRATE-SUPPLIED PLANTS

It has been shown that plants absorb ammonium and nitrate with varying ability depending in part upon the concentrations employed, the pH value of the nutrient solution, the presence of some free oxygen in the medium, and the carbohydrate content of the plant or its opportunity for new synthesis of sugars. Unless otherwise stated, the experiments chosen for the immediate discussion are concerned with the growth and metabolic responses exhibited by plants grown under reasonably favorable nutrient treatment for the form of nitrogen supplied. Unfortunately few, if any, experiments are available for discussion, concerning which it can be said that carbohydrates or opportunity for their synthesis may not have been a limiting factor in the utilization of ammonium nitrogen and in the growth of the plants. However, carbohydrate analyses which are in some cases available help materially in an understanding of the results obtained. It may be well to remind the reader that the experiments to be considered were conducted in glasshouses. These structures, even when in excellent condition, shut out 20 per cent of natural sunlight and often very much more (244).

It has been shown that, in the process of protein synthesis from nitrate, nitrite and ammonium are found in the plant in successive stages. Obviously on a theoretical basis, ammonium should be more rapidly assimilated by the plant, therefore, than nitrate. Under conditions of a culture medium favorable, respectively, for ammonium and nitrate, this has been found to be invariably true if carbohydrates have been present in sufficient quantity for synthesis of amino acids or other forms of elaborated nitrogen, and for detoxication of absorbed ammonium through the elaboration of asparagine or glutamine.

Tiedjens *et al.* (263, 264, 265) worked with tomato and several other species and supplied them with nitrate and ammonium, respectively, from the seedling stage to maturation of fruit. Young apple trees also were carried through several months of vegetative growth, receiving in some cases nitrogen only as ammonium and in other instances only as nitrate. Some of this work was in a degree duplicated in studies of the metabolic responses of apple trees at low temperature (159). When plant material was employed that contained an abundant carbohydrate reserve there was very much more rapid

synthesis of organic nitrogen from ammonium and much more rapid increase in volume of the plant than in the case of comparable cultures supplied with nitrate. Invariably this was accompanied by rapid depletion of sugars and starch. The organ first becoming depleted of carbohydrate reserves varied with the kind of plant, depending of course upon the seat of initial amino acid synthesis. In fruit trees this is mainly in the rootlets and it was in the root system that there occurred first the most striking decrease in nitrogen-free reserves. This was apparent earliest in the ammonium-supplied trees although the cultures receiving nitrate decreased in carbohydrates materially as compared to similar trees lacking an external nitrogen supply. The latter steadily increased in concentration of starch (*cf.* 73). Also the total organic nitrogen, amino acid and determined amide was very much higher in the roots of the trees receiving ammonium as compared to those receiving nitrate. The trees of the cultures lacking an external source of nitrogen were extremely low in concentration of nitrogen and contained little more than traces of the simple soluble forms mentioned.

In tomato plants and in the other herbaceous species under investigation there was, with continuous ammonium nutrition, a comparatively low carbohydrate content in all parts of the plants as compared to others receiving nitrate. The carbon skeleton of a carbohydrate or derivative is clearly essential for the synthesis of any organic form of nitrogen and it is, therefore, not surprising that with relatively rapid synthesis of organic nitrogen rapid consumption of carbohydrates occurred. The assimilation of organic nitrogen involves not only utilization of carbon in the amino acid or protein molecule but involves also marked increase in rate of loss through respiration (*cf. Nitrate Reduction*).

Except in midsummer under nearly ideal light conditions, the carbohydrate reserves of plants continually supplied with ammonium tended to become very low (263, 265, 154). This was associated with a soft, dark green, succulent growth and relatively unfruitful plants. These responses can scarcely be considered to be peculiar to ammonium nutrition for identical responses occurred during winter months in the case of nitrate-supplied plants. They were clearly associated with a condition of carbohydrate deficiency; at least carbohydrates were deficient in the sense that their concentration was inadequate for formation in abundance of the carbo-

hydrate derivatives, lignin and cellulose, the major constituents of the mechanical tissues of plants. Blossoming was seldom entirely eliminated but many of the flowers or developing fruits abscised, as is commonly the case when plants are deficient in carbohydrates. It may, of course, also occur if plants are deficient in organic nitrogen (*cf. GROWTH IN RELATION TO AVAILABLE NITRATE*).

The cultures concerned were in all cases supplied with a flowing nutrient solution or were given daily or twice daily applications. Under these conditions even a very dilute solution containing ammonium resulted in extremely rapid organic nitrogen synthesis. A very low ammonium content in the plants was apparently adequate for new synthesis of amino acids. This was in contrast to nitrate-supplied tomato plants which apparently required, at least under the experimental conditions obtaining, a very high concentration of nitrate in their tissues. Otherwise, reduction of nitrate to ammonium was seemingly inadequate in amount or rate to maintain sufficient synthesis of organic nitrogen for vigorous vegetative growth (*cf. 105*).

That these results in general are not peculiar to the location of the experiment or type of solution employed is obvious. Mevius and Engle (63, 130, 131) stress the importance of opportunity for carbohydrate manufacture and note the fact that available ammonium must be less in the winter under unfavorable light conditions than during the summer months. They feel that their analytical data, especially in its relation to seasonal conditions, substantiates the views of Prianischnikov in showing that carbohydrates, or opportunity for their synthesis, is essential for amino acid formation from ammonium and detoxication of ammonia through amide formation.

In connection with depletion of carbohydrates in the tissues of ammonium-supplied plants, the results of Holly *et al.* (82) are interesting. Owing to circumstances which were unavoidable, cotton plants during the later stages of vegetative development received less ammonium than they were capable of metabolizing so that in effect his plants were changed periodically from plus-ammonium to minus-nitrogen culture, to plus-ammonium, etc. Unfortunately, his carbohydrate analyses include only sugars which fluctuate materially with minor changes in environment but they showed a tendency to increase under the conditions of intermittent

ammonium nutrition and there occurred abundant boll development. He emphasizes the fact that with conditions favorable for carbohydrate formation, ammonium was much more rapidly converted into organic nitrogen than was nitrate.

Prianischnikov has repeatedly stressed the importance of carbohydrates in nitrogen nutrition in his work with seedlings and, in a recent paper (197) concerning the ammonium and nitrate nutrition of plants carried to maturity, he suggests that ammonium-supplied plants be occasionally deprived of all external nitrogen supply not because of any factor directly inherent in the nutrient material itself but rather to permit carbohydrates to accumulate.

The writer has frequently used ammonium nutrition of plants in sand culture in the greenhouse in preparing them for other experimental work, solely because more rapid responses can usually be obtained in this manner under conditions of adequate light; but for best results, it is absolutely essential to observe the quality of growth of the plants and when the current growth becomes unduly succulent, which invariably means carbohydrate deficiency, to omit all external sources of nitrogen and thereby permit carbohydrate reserves to become replenished, after which ammonium may be added again. It may be recalled that ammonium, excepting in very acid plants (cf. *Ammonium Storage*), is not stored in quantity, as nitrate often is (cf. *Nitrate Storage*). Accordingly, with ammonium nutrition it is possible to maintain, by observation of the quality of plant growth, a nice adjustment of protein and carbohydrate synthesis, the objective being to avoid a deficiency of either. With nitrate nutrition plants often accumulate such an enormous nitrate reserve in their tissues that under unfavorable light conditions a month or more may be required, with no external nitrogen supply, for disappearance of nitrate through reduction and assimilation.

From another viewpoint the desirability of avoiding carbohydrate deficiency is indicated by an interesting result reported by Beaumont *et al.* (12). They supplied various grasses and clovers with ammonium sulphate under sterile conditions in a complete water culture with no aeration. The tops of the plants were enclosed in clear glass lamp chimneys, the non-sterile controls presumably being likewise enclosed. These conditions are cited not in point of adverse criticism, for ideal environmental conditions could

not easily have been maintained and at the same time have kept the cultures sterile, but because the conditions are highly significant in that the opportunity for carbohydrate synthesis was limited by limited light, and the detoxication of ammonia was presumably seriously impaired through lack of aeration and consequent limited synthesis of amide (*cf.* 26, 111, 135, 235).

Although in addition to nutrient supply the environmental factors mentioned played an important rôle, it is significant that in the unsterilized solutions the roots decayed whereas roots of plants in the sterilized cultures did not decay. They suggest that the decay of roots was due to carbohydrate depletion coupled with accumulation in the roots of unassimilated nitrogen from ammonium sulphate, thus making the root tissue susceptible to the attack of organisms causing decay.

Clark and Shive's (37, 38) work on tomato, although already considered in connection with absorption, is of unusual significance in that the plants were grown in flowing cultures with adequate aeration and careful control of pH values. This technique was also followed even during the periods of the actual absorption tests. These results would indicate that, directly or indirectly, the pH of the nutrient medium limits assimilation rather than absorption, at least within the pH ranges employed (4, 5, 6 and 7). At these several hydrogen ion concentrations there was found in the roots, on a percentage of fresh weight basis, almost exactly the same concentration of nitrate. The ammonium, as recorded, allowing for glutamine hydrolysis that undoubtedly occurred with the analytical technique employed (39, 199, 276), indicates no deficiency of this ion in the roots at any pH value. They conclude that each ion was most rapidly absorbed when most rapidly assimilated. Their further results on analysis are difficult of interpretation as the plants were supplied with both ammonium and nitrate. Later work by Clark (39) on the composition of the tomato plant will be discussed in detail.

Davidson and Shive (47, 48) grew young peach trees in sand culture in two series of treatments, in one of which the cultures received nitrogen only as ammonium, in the other, only as nitrate. The nutrient solutions in both series were applied at pH 4 and 6. Constant renewal of cultures was employed and excellent control of pH obtained. Nitrate was limited exclusively to the roots except

in the pH 6 cultures. The trees of this group absorbed nitrate relatively slowly but it was found in both roots and tops indicating that the pH value of the culture medium limited assimilation rather than ability of the plant to take in the nitrate ion. The ammonium situation was essentially the same as that found by Clark and Shive for tomato (37, 38). The pH value of the solution seemed directly or indirectly to limit assimilation rather than intake of ammonium. Between the two better lots, the trees of the ammonium cultures at pH 6 and the nitrate group at 4, there was practically no difference in volume or quality of growth, it being excellent in both cases. It is of interest that the stems of both groups were very closely similar in percentage of total organic nitrogen and the fractions determined, including cyanogenetic material (cf. 209). The function of the cyanogenetic fraction is not clear and the concentration was low.

On the other hand, the roots, the principal seat of initial organic nitrogen synthesis in peach, were very much higher in elaborated nitrogen in the ammonium supplied trees at pH 6 than in the nitrate group at 4. This was owing mainly to a higher protein content. The pH 4 cultures which received ammonium characteristically made little growth, but they were high, rather than low, in total organic nitrogen. The detailed fractions furnish no explanation. The trees which received nitrate at pH 6 exhibited root and top growth that was not greatly less than that of the ammonium and nitrate series at pH 6 and 4, respectively. The most striking feature was the comparative deficiency of soluble organic nitrogen in the roots of the tree receiving nitrate from the less acid solution which, coupled with the appearance of nitrate in the tops, would seem, as already mentioned, to indicate limited ability to reduce nitrate.

In so far as possible, a technical discussion of methods of chemical analysis of plant tissue has been avoided in this review. However, it seems essential to point out here that the several experiments cited, including those with which the writer has been associated, although they have yielded certain proximate information on the amounts of total organic and protein nitrogen, include serious inaccuracies with respect to the determinations of ammonium and amide (cf. 39, 199, 276, 270). This is owing to the fact that boiling plant extracts at ordinary pressure hydrolyses the amide group of glutamine, resulting in a greatly increased ammonia value that

is, of course, spurious. Moreover, the amide-free residue of glutamine forms pyrrolidone carboxylic acid. This compound, which includes the original amino group of glutamine, yields no amino nitrogen by the usual Van Slyke procedure (36, 276). Nitrate-supplied plants, at least of tomato, contain relatively little glutamine or ammonia and while the fractionation of nitrogenous groups in the boiled extracts of such plants is probably approximately correct, the so-called "combined ammonia," referred to by Tiedjens (265) in the case of his ammonium-supplied plants, was presumably the product of hydrolysis in whole or in part of the amide group of glutamine.

There are unfortunately a considerable number of papers concerning the metabolism of ammonium and nitrate that are based upon analyses of plant tissue dried gradually in ovens at low temperatures such as 45° or 65°. This results in autolysis, as Chibnall has shown (30). Such analytical results do not represent or even remotely approach the conditions actually occurring in the plant. Drying finely minced tissue rapidly, brittle and practically to constant weight in the first two hours, at a temperature of about 80° in a forced draft very largely eliminates autolytic changes in the simpler nitrogenous constituents (272). However, results recently obtained in the writer's laboratory indicate that the amount of insoluble or "protein" nitrogen, at least in some plant material, varies with fresh tissue as compared to tissue dried rapidly at 80°. This is not an argument for or against the use of fresh tissue in determination of the heterogeneous fraction called protein; but the method chosen and the coagulent employed should be considered in comparing the yield of "protein" obtained by the two procedures.

The careful study of analytical technique carried on cooperatively in the laboratories of Vickery and Chibnall (276) has resulted in methods of analysis which, when applied to experimental plant material, have furnished the most complete information yet obtained on the nitrogenous and related organic acid metabolism of ammonium- and nitrate-supplied plants. Clark (39, 275) grew tomato plants in sand culture with continuously renewed complete solutions at pH 6.7. One solution contained only calcium nitrate as a source of nitrogen; another, an equivalent amount of ammonium sulphate; a third contained one-third as much ammonium sulphate. These plants, one month old at the initiation of his ex-

periments, were grown for 49 days with a relatively limited opportunity for carbohydrate synthesis as the glasshouse employed was severely shaded by surrounding trees. This is clearly reflected in the extremely low percentage dry matter in the stems of all the plants of the several cultures. The differences in percentage dry matter are small and it should be borne in mind that soft tomato plants of the type described fluctuate materially in water content (154). The plants were of a soft succulent vegetative type in all cases. The undoubtedly low carbohydrate content of his plants was an influencing factor which should be considered in evaluating the growth and metabolic responses obtained.

However, the fresh weight of the nitrate-supplied cultures was over twice that of the ammonium groups. There was in this respect no significant difference between the cultures receiving the high and low concentrations of ammonium. Probably carbohydrates were less a limiting factor in the nitrate- than in the ammonium-supplied plants (*cf.* 263, 265, 159). There certainly was the usual indication of relatively rapid assimilation of the ammonium ion in that the stems of the plants of the series receiving the high level of ammonium were over twice as high in concentration of soluble organic nitrogen and proportionately low in protein as compared to the nitrate series. With a higher carbohydrate reserve a greater degree of condensation of amino acids to protein would be anticipated. Tiedjens (265) obtained a similar response with tomato. This is not peculiar to ammonium nutrition but is intimately correlated with the concentration of carbohydrates (*cf. Synthesis of Storage Proteins*).

It is probably significant that the roots of the several series were practically the same in percentage content of soluble organic nitrogen. In tomato the initial stages of ammonium and nitrate assimilation take place mainly in the aerial organs of the plant. It will be recalled that peach trees (48) exhibited similar differences in organic nitrogen concentration but it was in the roots, the organs of initial synthesis of amino acids, rather than in the tops where there was little or no difference in quantity or quality of nitrogenous materials.

Clark's determinations of ammonium contained in the plants are probably the most accurate available for tomato and they indicate that this plant contains little ammonium even when abundantly

supplied. Moreover, the concentration is greater in the aerial organs than in the roots. As usual, the plants supplied with nitrate accumulated it in enormous concentrations especially in the stems (*cf. Nitrate Storage*).

As already mentioned, the soluble organic nitrogen was extremely high in the stems of the ammonium series. Within this fraction much larger percentages of glutamine and asparagine nitrogen were found than in the nitrate group. As a result, total amino nitrogen and known soluble organic nitrogen composed large percentages of the soluble organic nitrogen in the plants receiving ammonium. In contrast, the major part of the soluble organic nitrogen in the plants supplied with nitrate was composed of unknown soluble organic nitrogen. It may have included in large part polypeptides or related compounds as none of the determinations made included materials of this general group.

One of the most pronounced contrasts between plants of the ammonium and those of the nitrate series was in the concentration of organic acids in their tissues. The concentrations of the individual acids, oxalic, malic, and citric, were all higher, respectively, in the plants receiving nitrate than in those supplied with ammonium. His data show that both the known and the total acids were much more abundant in the plants of the nitrate than in those of the ammonium cultures. Moreover, the percentage of the total acids composed of the three known acids was much greater in the former than in the latter case in corresponding organs. Unknown acids not only formed a large fraction of the total acids of the ammonium-supplied plants, but also were present actually in relatively large amounts per unit of tissue, except in the roots of the plants that received the higher level of ammonium.

Ruhland and Wetzel (212), it will be recalled, found that in *Begonia* oxalic acid was relatively high when nitrate was supplied, relatively low when ammonium was the external source of nitrogen. Clark suggests that these results, in harmony with his, and the relatively low concentration of total acids may have been correlated with the available carbohydrate reserves which, under comparable conditions, seem to be invariably lower with abundant available ammonium than with nitrate freely supplied. It would be of interest to know the organic acid situation in tomato plants high in carbohydrates that had been grown for some time with no exter-

nal supply of nitrogen. The stems of such plants as frequently observed by the writer commonly exhibit an abundance of calcium oxalate crystals in the mature relatively acid parenchymatous tissues. Schneider (218) also found that calcium oxalate accumulated in nitrogen-deficient plants. Clark suggests the remarkably high asparagine and glutamine content of the plants of the ammonium groups may have been formed at the expense of organic acids, or their precursors, with consequent reduction in the amounts of organic acids in tissues rich in amides.

It is further suggested that the data do not warrant an assertion that the unfavorable effect of a high concentration of ammonium nitrogen in the tissue was due to any "toxic" effect of this ion. The relatively low ash content found to obtain in the ammonium-supplied plants (*cf. Non-Nitrogenous Ions*), coupled with the low concentration of organic acids, is indicated as being a possible causal factor contributing to the lesser growth of the plants of the ammonium groups. The suggestion concerning ash is in harmony with Prianischnikov's (197) work on beneficial effects of added calcium in ammonium nutrition, and the apparent importance of organic acids is not in conflict with the view that carbohydrates are essential for detoxication of ammonia through amide synthesis. Rather, the function of carbohydrates in this connection is seemingly made more specific, suggesting very strongly that the apparent rôle of carbohydrates in amide synthesis is indirect. However, carbohydrates are directly or indirectly necessary for organic acid formation and, as has been frequently shown by many experiments, formation of amide and detoxication of ammonia fail when the carbohydrate reserves of the plant become depleted.

In this connection the importance of asparagine has been especially emphasized in the literature but the work of Greenhill and Chibnall (69) and Vickery *et al.* (278) make it apparent that glutamine should not be assigned a minor rôle. The former workers found that, when perennial rye grass was supplied with ammonium sulphate in conjunction with abundant calcium, a white exudation appeared on the upper half of the blades. The exudate consisted almost entirely of glutamine.

The latter group of workers supplied beets in the open field with frequent applications of ammonium sulphate. Under these circumstances the glutamine content of the root tissue rapidly increased

although the asparagine content was not affected. At the final stage, before definite injury to the plants occurred, a glutamine concentration of 5.4 per cent of the dry weight of the root tissue was attained. There was little effect upon the composition of the tops. Only after severe damage had occurred did the concentration of glutamine in the tops increase (*cf. Nitrate Reduction in Roots*) (49).

These experiments and Clark's (39) show that the quantity and kind of amide present in the plants concerned was directly or indirectly dependent upon the external source of nitrogen. Mothes (135: 472), in discussing the two amides, remarks that "in Russian sugar beets asparagine occurred in large quantities while in German sugar beets glutamine was found." It is not clear whether he implies a difference owing to variety or that induced by unlike environment. Both tomato and beet synthesized large amounts of glutamine when ammonium was supplied whereas with nitrate nutrition (tomato) amide nitrogen was low. Many examples have been cited of cases where asparagine fulfilled the function of ammonia detoxication (*cf. Detoxication of Ammonia*). Glutamine apparently performed this rôle in tomato, and asparagine in beet appeared in quantity only as the plants approached the stage of definite tissue injury.

In considering their experimental results, Vickery *et al.* (278) emphasize the fact that the increase in soluble nitrogen of beet root tissue, on treatment of the soil with ammonium sulphate, was equivalent to the glutamine nitrogen rather than to the glutamine amide nitrogen. Although they feel that the close quantitative agreement in their experiments may have been fortuitous, they emphasize the fact that it was apparent that both nitrogen atoms of glutamine functioned in detoxication of ammonia. Simple dehydration of the ammonium salt of glutamic acid might account, as they point out, for the amide group. It would seem obvious that the precursor of glutamine is a nitrogen-free carbohydrate derivative but the manner of function of the amino group is obscure. An explanation will necessarily await additional experimental evidence.

NITRITE NUTRITION

It has been pointed out that nitrate under usual nutritional conditions is not found in the plant at all, or only in very minute quan-

tity. Certain experiments have been described, however, by means of which the presence of nitrite can be detected in plants supplied with nitrate during a period of rapid reduction, nitrite being a transitory intermediate product in the formation of the end product ammonia (*cf. Nitrate Reduction*). It would seem reasonable, therefore, to expect that nitrite under suitable conditions would be readily absorbed and assimilated by plants. In the earlier literature there are records of occasional tests of sodium nitrite as a source of nitrogen. The quite contradictory results are of little more than historical interest, there being no record of the pH value of the nutrient medium and no provision for maintaining a constant concentration of solutes in the substrate.

One of the first extensive investigations on nitrite nutrition in which reasonably favorable cultural technique was employed was reported by Mevius and Dikussar (54, 132). Without describing all of their earlier work in detail it will be sufficient to summarize the more recently reported results.

Corn was grown in flowing water cultures, all of the cultures being supplied with the usual essential elements. It was found that with sodium nitrite as the sole external source of nitrogen, concentrations of nitrite as high as 200 mg. of nitrogen per liter of nutrient solution, at a pH of 7, could be employed without injury to the plants if light conditions were favorable for rapid CO_2 assimilation. During most of the period of their experiments, however, they found that 50 mg. of nitrite nitrogen per liter gave the most desirable growth responses.

With excessive applications of nitrite under unfavorable light conditions there was a very rapid increase in soluble organic compounds of nitrogen but little condensation of amino acids to protein, and amide nitrogen accumulated. It is apparent that these responses are strikingly similar to those already recorded for low-carbohydrate plants supplied with high concentrations of ammonium. Much as in the case of ammonium-supplied plants, the more acid the nutrient medium the lower the concentration of nitrite had to be to avoid injury to the plants.

In comparison of cultures supplied with nitrate, nitrite and ammonium, respectively, under pH conditions of the culture solution reasonably favorable for each, they observed the usual tendency for nitrate to accumulate in the plants, whereas nitrite, like ammonium,

occurred only in very low concentrations, presumably being rapidly reduced to ammonium with subsequent assimilation at the expense of carbohydrate reserves. This is suggested by the distribution of organic compounds of nitrogen appearing in the plants receiving nitrite. In both roots and tops the highest percentage of amide nitrogen as well as total soluble organic nitrogen was found in the plants of the ammonium series. The nitrite group was only slightly lower and in striking contrast to the values recorded for the nitrate-supplied plants. A relatively high proportion of the elaborated nitrogen of the plants furnished with nitrate was present as protein, and amino acids rather than amide nitrogen predominated.

Fraps and Sterges (66) grew cotton seedlings for 18 days in water culture with a complete nutrient solution containing nitrogen only as nitrate. They used Shive and Stahl's (234) technique of continuous renewal and aeration of the water cultures. At the end of that time, after the roots were washed, some of the plants were shifted for 24 hours to a similar solution containing nitrogen only as sodium nitrite. The initial pH of the solutions was approximately 6 and at the end of the absorption test was slightly less acid, about pH 6.2. There was slightly greater absorption of nitrite than of nitrate nitrogen. It is probable that somewhat more rapid absorption of nitrate would have occurred from a solution at about pH 5 (*cf. The pH of the Nutrient Solution*). They also made the observation that with both sources of nitrogen more than six times as much was absorbed in the 24 hour period as in the first six hour interval, though the time was only four times as long.

Effects of the pH of the culture solution on the utilization of nitrate have already been discussed but the results of Fraps and Sterges (66) are of additional interest in that they afford a basis for comparison with the pH values found most favorable for nitrite nutrition. They found that corn made the greatest growth with nitrate when the nutrient solution had a pH of 3.9 to 4.1, the least growth at pH 6.4 to 7.7, whereas with nitrite the lowest growth was at pH 3.9 to 4.1 and the highest at pH 6.6 to 7.7. A similar response was exhibited by cotton and oats. However, the best growth of the nitrite-supplied plants under the most favorable pH conditions employed, being much the same as those considered optimum by Mevius and Dikussar (54, 132), was much less than that of the most vigorous plants which received nitrate. Judging from their

plant descriptions this may have been associated partly with iron deficiency. The high pH values essential for nitrite nutrition necessarily make it difficult to maintain in the culture medium a source of soluble iron that is adequate for some plants. (210).

Their nitrification work with soils, which may be briefly mentioned, indicates that nitrite is more likely to be produced in alkaline soils than in soils with a lower degree of acidity. Apparently the soils in which nitrites are likely to be produced are those in which they are not likely to be toxic in small amounts.

ABSORPTION OF ORGANIC COMPOUNDS OF NITROGEN

The proteins and related compounds are obviously essential for plant growth. It has been shown that plants can synthesize proteins from inorganic salts of nitrate, nitrite and ammonium. The synthesis to proteins is inadequately understood but, nevertheless, it is clear that the chemical transformation does not take place in one step. Some of the intermediate products in this synthesis are known, however, and have been indicated in the preceding discussions. They include semi-amides, asparagine and glutamine along with a large number of amino acids. The latter especially are formed both in the synthesis of new proteins and in the breaking down of storage proteins through hydrolysis as, for example, in the germination of seeds. It is well known that many of these compounds are found in soils where they are formed through the cleavage of the proteins of decaying organic materials (282, 107, 22).

It seems reasonable to think, therefore, that if the plant absorbs such compounds as amino acids from a soil or nutrient solution, its utilization of them, if in equivalent amounts, must occur just as if they had been synthesized in the plant from nitrate, nitrite or ammonium. The nitrogen of amino acids and related compounds represents a greatly reduced form of nitrogen. As already pointed out, it necessarily follows that their synthesis from inorganic nitrogenous nutrients can occur only following expenditure of energy apparently obtained in large part if not entirely through oxidation of reserve carbohydrates or their derivatives (*cf. Nitrate Reduction*). It would seem, therefore, that the amino acids of the soil, or related compounds, should be a singularly efficient source of nitrogen.

That plants can utilize various compounds of organic nitrogen when supplied externally through the cut ends of stems or petioles has already been indicated. Klein and Linser (104), for example, placed the ends of cut stems of tobacco plants in a nutrient solution containing proline and greatly increased the nicotine content of the plants as compared to the controls. By injection into the hollow stems of growing plants they obtained an increase in the betaines on supplying glutamic acid, ornithine, proline or hexamethylenetetramine (101). Many other results of this type might be cited (*cf.* 18, 19, 140). These results, while showing that plants can metabolize certain compounds of organic nitrogen when supplied externally, can scarcely be considered in the same category as absorption by intact roots.

Some of the earliest work on the absorption of organic compounds of nitrogen was reported by Hutchinson and Miller (83). They grew pea plants in sterile cultures usually with two plants per culture and determined by analyses of the plants whether or not an increase in absolute amount of total nitrogen occurred. Proceeding on this basis, they listed certain organic compounds that were good sources of nitrogen, others that were doubtful or even toxic. Schreiner and Skinner (219), at about the same time, tested the effects of a very large number of compounds of organic nitrogen upon the growth of wheat seedlings, employing compounds they had found to be present in soils. They changed the solutions of their water cultures every three days and although the plants were not grown under strictly sterile conditions great care was taken in that the solutions were changed every three days and the plants shifted to sterilized receptacles at that time. They obtained increased growth of their plants with additions, respectively, of creatinine, creatine, nucleic acid, xanthine, guanine, histidine, arginine and asparagine. In the case of nutrient solutions containing nitrate, added compounds of organic nitrogen increased growth from 11 to 23 per cent as compared to the cultures receiving nitrogen only as nitrate. Analyses of residual solutions showed definite removal of organic compounds of nitrogen from the cultures. In certain cases removal was faster in the presence than in the absence of nitrate.

Unfortunately, the matter of aeration and of the pH of the culture medium was not at that time appreciated but the work of Schreiner and Skinner definitely established the fact that many

organic compounds of nitrogen may be absorbed by intact roots. Whether or not increased growth occurs with additions of amino acids will of course depend in part upon the degree of nitrogen deficiency of the plants being supplied with the material in question; more specifically, probably, with the content of the compound in the plant that is being furnished to it externally. For example, a carbohydrate-deficient plant containing a high concentration of proteolytic products and asparagine would scarcely be expected to absorb asparagine as freely as a plant of the same kind containing less organic nitrogen and an abundant carbohydrate reserve. It should also be remarked that seedlings which have been used for the most part in this type of work are notably high in proteolytic products. Also the use of sterile cultures, although clearly desirable, has tended undoubtedly to accentuate the already high amino acid content of seedlings, owing to the limited light available in the flasks often employed for enclosing the aerial organs. The lack of aeration of the culture medium, as already shown, greatly modifies the external responses and metabolism of most plants.

The intention is not to minimize the value of much careful work with sterile cultures wherein ideal conditions of environment are difficult if not impossible of attainment. It should, nevertheless, be emphasized that under usual field ecological conditions there may well occur much greater utilization of amino acids than the results of present cultural technique indicate.

Some of the work concerning utilization of nitrogenous compounds diffusing from the root nodules of legumes supplies considerable pertinent information. Lipman (109) demonstrated that oats, a non-leguminous plant, absorbed nitrogenous compounds derived from a host legume, in this case the pea plant. Sand culture was employed and the two kinds of plants were separated from each other by an impermeable partition and other similar groups by a permeable partition. The peas exerted a definite beneficial effect on the growth of oats where diffusion between the two plants was possible. The effects were so marked even when the plants were very young that decay of cortical root tissues could scarcely have been a significant contributing factor.

Recently, Virtanen (279, 280) has reported the results of extensive experimental work. In typical experiments his technique has been to grow one leguminous and one oat plant together in flasks

under sterile conditions except for the presence of specific legume nodule bacteria. In some cases the plants were entirely enclosed in glass receptacles; in others, bottles with two or more necks were employed out of which emerged through a cotton plug the tops of the plants which were grown in sand or agar culture. He found, as did Lipman, that legumes with nodules supplied nitrogenous material which permitted growth of oats. He found that the materials diffusing from the root nodules were principally amino acids. Neither ammonium nor nitrate was present. Barley, for example, made excellent growth when its only external source of nitrogen consisted of the organic compounds of nitrogen furnished by the host legume. It is of interest that in other experiments in which barley was grown under sterile conditions independently of legumes, asparagine proved to be one of the best of the organic compounds tested and was apparently absorbed, as was aspartic and glutamic acid, without any material shift in the pH of the nutrient media. Aspartic acid was associated with good growth of various leguminous plants but did not seem to be utilized by barley and certain other grains under the experimental conditions. Employing somewhat similar technique and sterile conditions, Klein apparently found considerable variation in the absorption of amino acids, glycine and alanine seeming in general to be less freely utilized than asparagine, aspartic and glutamic acid (98).

Certain experiments concerning the utilization of urea are of interest. Pirsche (175) found that the roots of plants which had been supplied with this compound in water culture exhibited greater urease activity than roots of similar plants supplied with the usual forms of inorganic nitrogen. Pirsche considers that the cleavage of urea to ammonia may take place in the plant independently of bacterial action. Yamaguchi (298), in studies of the responses of corn seedlings to urea supplied in sterile cultures, concluded that urea may be absorbed as such. He found no ammonium in his cultures but records an initial pH value of the nutrient medium of 4.6 which shifted to 5.2 to 5.5 and then again became more acid, 3.6 to 3.8. In Pirsche's experiments the change in pH was towards increased alkalinity. Yamaguchi's analytical technique (Xanthodrol method) would not, however, distinguish between urea and ureides. He reported that urea (ureide?) was present in the aerial organs and in the guttation water of the leaves.

Klein and Taubock (102, 103), in experiments with corn and beans in sterile culture supplied with arginine, indicate that this amino acid may be absorbed by plant roots but that owing to the presence of arginase in the plants there is rapid decomposition of it to urea and presumably to ammonia. The matter of metabolism of directly absorbed organic compounds of nitrogen remains, however, an almost uninvestigated field of research. It should be an extremely profitable one if considered in relation to any concentration and pH changes of the culture medium in their interrelation to the initial and subsequent nitrogenous and carbohydrate metabolism of the plant. Finally, attention may again be called to the earlier investigations of Schreiner and Skinner wherein a combination of nitrate and organic compounds of nitrogen gave better growth responses than either employed alone. Of course, this may have been a matter of a favorable change in pH of the media brought about by addition of the "physiologically alkaline" nitrate salt to the solution containing an amino acid. Nevertheless, it is of interest that they suggest that if a soil is liberally supplied with all the building units for proteins (amino acids etc.), it is conceivable that good plant growth might result without nitrate. If only a limited amount or assortment of units were present in the soil, then the plant might presumably require nitrate with which to synthesize the lacking units. However, they point out quite reasonably that plant enzymes may be able to transform one or more of these units into other or closely related units.

A few additional references are given that are of interest but merely listing compounds of organic nitrogen that have been reported to have given good, bad or indifferent results means little for reasons already discussed (1, 2, 11, 12, 253, 255).

GROWTH IN RELATION TO AVAILABLE NITRATE

Abundant evidence has been presented showing that ammonium may be absorbed and assimilated by plants with extraordinary rapidity. Although we know little about the direct absorption and utilization of organic compounds of nitrogen it may well be of considerable importance in many fertile agricultural soils. Under usual commercial field conditions the principal source of nitrogen is undoubtedly nitrate, nitrite being but a transitory product. Nitrogenous materials very quickly change to nitrate in most tillable

soils so that no matter what kind of nitrogen-containing fertilizer is applied the plants absorb mainly nitrate (205, 238). There are some exceptions where heavy applications of ammonium sulphate are applied in a localized area about the plant. But where ammonium is found as a more or less permanently present constituent of a given soil, it is usually owing to lack of nitrifying organisms and is correlated with poor aeration or a low pH value of the soil, or both; and these conditions, as already have been shown, practically eliminate the possibility of efficient utilization of ammonium except by certain specialized types of plants (129, 148, 215).

Studies of effects of a lack of available nitrogen have been in continual progress in fertilizer plots at the Rothamsted Experimental Station for nearly 100 years (215). Considerable interest must, therefore, be attached to the responses exhibited by the plants of the several levels of fertilization. The plants lacking adequate nitrogen are described as being stunted in growth and yellowish green. The red pigments of anthocyanins are often conspicuous on the foliage. Apples, when present, are few and limited in size but very highly colored with red for the variety. These characteristics are too well known to require further comment.

Also, the plants are typically stiff and woody when nitrate is low, owing to thick cell walls and the formation of mechanical fibers, sclerenchymatous tissue, etc. These observations have been corroborated by Kraus and Kraybill (105), by Welton (288) and recently by Schneider (218). The leaves of such plants usually have a thick cuticle and epidermis; in short, the effect of lack of nitrogen on mechanical structure is to produce a relatively xeromorphic plant.

The fibrous roots of low-nitrogen plants are notable in that, although of small diameter, they are usually very extensive and branch and rebranch with many fine sublateral rootlets. This again is an observation so frequently recorded in horticultural literature that little more need be said (*cf.* 201, etc.). In this connection, however, may be recalled the striking increase in dry matter yield and in volume of roots recorded by Hamner in the case of the series of wheat plants deprived of nitrate (73).

Additions of nitrate to cereal plants exhibiting such symptoms were said by Russell (215) to have caused a marked and rapid increase in green color and volume of growth. On the other hand,

greater quantities of nitrate led to the development of large, dark green leaves which were often "soft, sappy, and liable to insect and fungous pests". The cuticle and cell walls of the leaves were thin and the development of mechanical tissues was limited, a result which has very frequently been associated with heavy applications of nitrate (105, 218, 288, 155, etc.).

Further effects of a large supply of nitrogen are recorded (215) as having retarded ripening. Plants receiving different amounts of nitrate were thus at different stages of their development at any given time, even though they were all sown on the same day. Those supplied with large quantities of nitrate continued vegetative growth for a longer period. Russell remarks further that seed crops, like barley, that should be cut "dead ripe," should not be supplied with much nitrate, but oats which are cut before being ripe can receive larger quantities. It was said, however, of all the cereal crops, that they produced too much straw if the nitrate supply was excessive and that the straw did not stand up well but was beaten down or "lodged" by wind and rain. Crops such as mangolds and potatoes also produced abundant leaf growth but not proportionately more root or tuber, etc.

In one case it was reported that at the Cheshunt Station the omission of nitrogenous compounds from the fertilizer mixture caused an increase of 11 per cent in yield. Wallace and Sylvester (283) found that lowering the nitrogen content in apples with accompanying increase in sugars by cultural treatments, or ringing, raised the vitamin C content of the fruit as much as 1.5 to 2 times. Obviously, an excess of available nitrate as well as a deficiency is undesirable, deficiency being simply a relative term, for, as Russell (215) indicates, a deficiency of available nitrate for oats might well be an excess for a crop like barley. These or similar results obtained over a long period of years can scarcely be discarded and, furthermore, are in harmony with an enormous amount of horticultural literature.

Without repeating in detail certain interrelations of nitrate assimilation and carbohydrate metabolism, the reader may be reminded that nitrate will not materially effect the growth responses of plants—that there must first occur organic nitrogen synthesis. This involves an endothermic reaction with oxidation of carbohydrates or their derivatives and a greatly increased rate of respiration

(*cf. Nitrate Reduction*). Clearly, new synthesis of organic nitrogen, if carried to excess, will tend greatly to deplete the carbohydrate reserves of the plant. Correlated with such a deficiency there can scarcely occur strongly developed mechanical elements, for lignins and cellulose are carbohydrate derivatives in their most condensed form. Peaches from trees heavily fertilized with nitrate are usually relatively late in ripening, comparatively acid and low in sugars (156). In extreme cases of over-fertilization with nitrate, although blossoms may appear, they, or their partially developed fruits, absciss. This probably is not owing directly to excessive organic nitrogen synthesis but rather to the resulting deficiency of carbohydrates. Likewise failure of plants to fruit abundantly when no nitrate is available is not due directly to a high carbohydrate reserve, although it is true that at least prior to senescence the high carbohydrate content is frequently associated with immobilization or condensation of proteinaceous materials (*cf. METABOLISM OF STEMS*). In that sense, cleavage and reutilization of protein goes on slowly unless carbohydrates are decreased by shading, pruning, high night temperatures with increased respiration, etc. (105, 154, 158).

The dominant limiting factor in the latter case is unquestionably a lack of available nitrogen; in the former, a lack of carbohydrates. This, in brief and in the opinion of the writer, is the essence of the relationships discussed by Kraus and Kraybill (105) in their studies of vegetative and sexual reproduction in tomato. There are, of course, all degrees of carbohydrate and organic nitrogen deficiency and an optimal status wherein, for the growth response desired, there is no deficiency of either, as indicated in Kraus and Kraybill's four classes. However, if the varied functions of carbohydrates and the many forms of nitrogen found in the plant are considered, it seems highly improbable that a ratio of significance can ever be obtained by dividing the total carbohydrate content of a plant by its nitrogen content. It is certain that Kraus and Kraybill never intended to make this and similar fantastic claims. Yet in practical agriculture the nitrogen nutrition of plants must be considered in relation to opportunity for carbohydrate synthesis in order to avoid a deficiency of either plant constituent.

There is an enormous amount of literature on the carbohydrate and nitrogen content of plants in relation to growth responses, much

of which is very contradictory. Aside from translocation phenomena and the fact that specialized organs have often been indiscriminantly included with all other parts of the plant making an heterogeneous sample of dubious significance, the biggest source of error lies in the fact that it has not yet been possible to express results on the basis of "protoplasmic mass," as do the animal physiologists. This has recently been brought to the attention of the writer in working with the pineapple plant. Often in a plant in which all the potential starch-storing cells were filled to capacity, macroanalysis, as expressed on the basis of percentage of green or dry material, indicated little starch. The reason was obvious: the plant contained an enormously high proportion of inert mechanical fibers, and analyses of expressed juice, although of interest, naturally did not include starch, the major carbohydrate reserve. Another plant type, relatively soft and succulent and with limited fibers, was by microscopic examination very low in starch, yet on a percentage of dry or green weight basis relatively more starch was indicated than in the case of the plant filled to capacity with this material. Of course, the absolute amount of starch or other material could be easily computed but concentration or quality of protoplasmic materials, not absolute amounts, is associated with quality of growth. Absolute amounts are correlated with size, not quality of plant.

Very recently it has been discovered that unsaturated compounds such as acetylene properly employed can induce sexual differentiation of floral organs almost at will (91). The carbohydrate and organic nitrogen content of the plant can vary within very wide limits and yet on treatment with acetylene differentiation occurs. The writer has recently had an opportunity to participate in experimental work involving the use of acetylene. As might be anticipated, the nitrogen (and CO₂ nutrition) of the acetylene-treated plants still remains as critical a factor as in the case of plants which differentiated without the acetylene stimulus. Fruits of high quality, of desirable texture, containing abundant sugar were produced only on plants containing abundant carbohydrate and proteinaceous reserves in their vegetative organs. Although the nitrogen and CO₂ nutrition of plants is apparently only an attendant factor in relation to sexual reproduction, the fact remains that years of agricultural research show beyond doubt that a serious deficiency

of either organic nitrogen or carbohydrates in the plant is often associated with failure to produce flowers, or, if flowers appear, fruits are abortive and absciss prematurely (*cf.* 28, 67).

Nitrogen nutrition may also greatly modify the quality of fruit even though neither carbohydrates nor organic nitrogen are low enough to prevent their eventual maturation (156). Bishop and Russell (16, 17, 216), in summing up the results of investigations of barley, show that no matter where a given variety was grown nor what the fertilizer treatment—and there were included different sources and amounts of nitrogen—there was, over a period of 10 years, a consistent correlation between the concentration of the individual proteins and the total protein nitrogen and carbohydrate content of the grain. A relatively low percentage of total protein nitrogen (high percentage of carbohydrates) in the grain was associated with a high proportion of salt soluble nitrogen and low concentration of hordein nitrogen. Conversely, when total protein nitrogen was high (and carbohydrates were low) in the grain, there was proportionately less salt soluble nitrogen and a high percentage of hordein nitrogen. The proportion of glutenin was practically constant (*cf.* 89). Although we know little about the factors directly responsible for sexual reproduction, it is obvious that nitrogen nutrition plays a dominant rôle in determining the responses obtained. Certain ecological factors are discussed in the following pages in their relationship to nitrate nutrition.

EFFECTS OF TEMPERATURE ON NITRATE NUTRITION

The term nitrogen deficiency, so commonly employed, is not strictly applicable in many cases to plants which are low in content of assimilated or organic nitrogen. They may exhibit all the symptoms so characteristic of plants commonly included in this category yet may contain very high concentrations of nitrate. Nitrate, as already pointed out, is a potential source of organic nitrogen that may be stored in many plants but it is effective only following reduction and assimilation, not before. In recent work with pineapple plants at comparatively high elevations, where during the winter months the temperatures became relatively low, the plants exhibited all the typical symptoms of "nitrogen deficiency" yet contained in the massive stump or stem as much and sometimes more nitrate than organic nitrogen. Clearly, the external supply of nitrate was ade-

quate and it was equally apparent that absorption and translocation were not limiting factors.

Similarly in tomato plants grown in sand culture at a temperature of 13° C. nitrate was absorbed instantaneously, in about five hours was present in high concentration throughout the plant and remained high; but nitrate was reduced and synthesized to organic nitrogen very slowly. The plants accumulated carbohydrates, especially starch, in large quantities and in appearance were almost identical with other plants that were grown with no external nitrogen supply but a temperature of 21° C. Rosa (211) found a similar response in tomato, and Werner (290) in potato, the external expression of carbohydrate deposition being in greatly increased tuberization in the latter case. Platenius (179), during periods of low temperature, found that carbohydrates and nitrate accumulated in celery. Robbins *et al.* (207) report much the same situation in cauliflower and many more similar cases may be found in the literature.

Carbohydrate accumulation is undoubtedly the result of decreased utilization of carbohydrates in organic nitrogen synthesis but also, as was found in peach trees, with a greatly reduced respiration rate at low temperature yet a remarkably high plane of CO₂ assimilation (161). High temperatures (35° C.), on the contrary, were associated with nearly negative CO₂ exchange in peach under the same light conditions that resulted in carbohydrate increase at low temperature. Vigorous nitrate assimilation and rapid respiration continued until carbohydrates became depleted and protoplasm seriously injured. Associated with carbohydrate deficiency, the plants at high temperature, as might be anticipated, were weakly vegetative, soft and succulent, and exhibited all the symptoms so characteristic of plants lacking an adequate nitrogen-free reserve. The seat of nitrate reduction and initial amino acid synthesis in the fruit trees mentioned was limited as usual to the fine rootlets but metabolism was apparently similar to that already recorded.

EFFECTS OF DAY-LENGTH ON NITRATE NUTRITION

In conclusion, the much discussed problem of the effects of length of day and night may be mentioned in its relation to nitrate nutrition. The subject has been adequately reviewed by Burkholder (25) and the writer is heartily in accord with the view expressed. As he points out, the direct causal factors associated with floral

differentiation remain to be determined. The most extensive investigations under the most carefully controlled conditions of environment have been conducted by Arthur *et al.* (4). They list the responses of a large number of plants but in their chemical analyses unfortunately did not determine nitrate which under short day conditions may accumulate in very high concentrations and in some plants in amounts equal to or greater than their content of organic nitrogen (60, 77, 152, 157, 304). Nitrate accumulation is not directly associated with cessation of vegetative growth—that occurs in many plants with initiation of flowering—for in Biloxi soy bean plants nitrate accumulated even though the day-length was such (6 hours) as to entirely prevent any sexual response (157). The high nitrate content is the result of inability on the part of the plant to reduce and assimilate the contained nitrate. Accompanying this response, carbohydrates, especially starch, usually accumulate in very high concentration unless the long nights are warm, when a decrease in carbohydrates occurs presumably because of a relatively high rate of respiration (*cf.* 290).

It may also be remarked here that amino acids and amide nitrogen were made to accumulate in short-day Biloxi soy bean plants by shifting the plants to continual darkness for several days—a typical proteolytic response (*cf.* SYNTHESIS AND HYDROLYSIS OF STORAGE PROTEIN). Following the period of darkness, some of the plants were returned to short-day conditions and yet even with eventual increase in carbohydrates condensation of amino acids to proteins was limited and there was no significant change in concentration of amide nitrogen. Apparently the short days (long nights) directly or indirectly limited not only the initial reduction and assimilation of nitrate but the later stages of synthesis to protein. Under variable conditions of temperature, including especially high night temperature, amino acids might, therefore, accumulate in short-day plants as the result of cleavage of stored proteins rather than through new synthesis from nitrate (unpublished results by writer).

Arthur *et al.* (4), in considering the interpretation of their plant analyses, segregated plants into groups on the basis of whether or not any flowering response was exhibited. Fruiting of tomato plants was "taken to mean the setting and continued growth of three or four fruits per plant" and, of course, in the same class were included other plants with many fruits that must have been vastly

different in quality of growth. They divided the total carbohydrate content of their plants by the total nitrogen content and the quotient, or "C/N ratio," as might be anticipated, showed no correlation with anything.

Other factors than the supply of carbohydrates and organic nitrogen in the plant obviously influence sexual differentiation, as is shown by the effects of acetylene already mentioned, but, nevertheless, there seems to be under many circumstances an intimate association of the direct causal factors for flowering with the proteinaceous and carbohydrate reserves of the plant, even though many different combinations of ecological factors contribute to their synthesis. Some plants at least, as strains of *Salvia* that ordinarily blossom only during a short photoperiod, can be made to bloom profusely under long-day conditions simply by limiting the external nitrogen supply and thereby permitting carbohydrates to accumulate (152). Later experiments have shown also that *Salvia* blossomed little or not at all under short-day conditions if a state of carbohydrate deficiency was maintained by shading or effected by the use of high night temperatures, the latter result presumably being due to high respiration rate during the long night. Borodina (20) reported that the external nitrogen supply influenced seeding of barley, and by limiting nitrogenous nutrients she induced flowering at a day length usually associated with vegetative growth. Any number of references might be cited showing that short-day conditions favor the formation of carbohydrate storage organs. Zimmerman and Hitchcock (304) found that heavy root storage in 6 varieties of dahlia was correlated with a short day, and nitrate accumulated in the leaves and stems of the short-day plants but was absent or present in only small amounts in the long-day plants. Maximov (127) obtained tuber formation in *Solanum demissum* in a short day but not in a long day. Tincker (266) observed carbohydrate accumulation under short-day conditions as did Moshkov (134). The former author suggests that carbohydrates accumulate because the plants blossom. This is not always the case, however, for if the day length is extremely short no blossoms appear on Biloxi soy beans yet carbohydrates accumulate in high concentration (152, 157, 60). Werner (290) goes a step further, emphasizing that short days, although giving in general the influences recorded, can give the opposite response with respect to carbohydrates if

high temperatures are employed. The rate of nitrate reduction and assimilation may be greatly modified also according to the osmotic concentration of the nutrient medium. With a high salt concentration the sweet pea plant absorbed nitrate freely but under such conditions the intake of water was greatly limited (*cf.* 128). This was associated with early maturation of cells and a greatly decreased rate of nitrate assimilation owing to the fact that only comparatively young cells containing abundant protoplasm were capable of active nitrate assimilation (164). Thus, carbohydrates were made to accumulate and a deficiency of them avoided to a considerable extent, even with unfavorable light conditions and the presence of abundant nitrate in the plant.

The organic nitrogenous and carbohydrate constituents of the plants can scarcely be considered to be the immediate cause of flowering (25, 105, 113, 144, 15, 8), but it is clear that intelligent steps may be employed in nitrogen nutrition to decrease or increase vegetative growth, and to hasten, delay or entirely eliminate flowering responses under many different conditions of environment.

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THE BOTANICAL REVIEW

VOL. III

APRIL, 1937

No. 3

GEOTROPISM IN PLANTS*

FELIX RAWITSCHER

Departamento de Botanica da Universidade de S. Paulo, Brazil

INTRODUCTION

In 1868 A. B. Frank introduced the word "geotropism" for the peculiar active force which is liberated by gravity within various parts of a plant. Since then the science of botany has accumulated an extraordinarily extensive quantity of data from observations upon this subject without having succeeded, however, in satisfactorily answering even one of the many questions which have been raised. The works of Frank, Sachs, Pfeffer, Noll, Charles and Francis Darwin and of many others during the latter part of the past century contributed toward the formulation and elucidation of our understanding of *irritability*, which today is the basis of most of the theories formulated in an attempt to explain the responses of living organisms to stimuli. In this conception every modification of external or internal conditions which induces a change in the behavior of an organism is known as a *stimulus*; and the changes induced thereby in the behavior of the organism are known as the *reaction*, or better, as the *end-reaction*. Several intermediate stages can be interpolated between the stimulus and the end-reaction and they are referred to as the *reaction-chain*. We know that there need not be any relation, at least not a simple quantitative one, between the magnitude of a stimulus and that of the end-reaction. The stimulus has been looked upon, therefore, as a liberating factor such as is known in physical systems and as is exemplified by the discharge of a gun or the complicated sequences of a shrapnel or torpedo explosion.

The conception of irritability is applied only to living matter, but it was certainly not the intention of the physiologists who intro-

* Translated from the original German by the editor and approved, with corrections, by the author.

duced the term into biology that it should imply that between the stimulus and the end-reaction there are basic laws other than those known in the realm of physics. None the less, there is a part of the reaction-chain which occurs within the living protoplasm and which today still defies physical analysis. Should an explanation become available some day, then we shall be able to speak of a concatenation of physical causes and effects rather than of one consisting of stimuli and reactions. It appears as premature, however, when certain physiologists, having this in mind, already attempt to discard the conception of irritability, as has been done in the field of phototropism from Blaauw's (3) time up to Du Buy and Nuernbergk (20). So far as geotropic phenomena are concerned, at least a part of the reaction-chain, as we shall soon learn, takes place within the living protoplasm under conditions which today are not yet well understood. Under no circumstances, then, can we discard the conception of irritability.

The subdivisions which today serve as a general basis in an account of geotropic phenomena are founded upon the nature of the end-reaction. If a curvature is involved, as is true of the best known cases, we speak of a *tropism* in the narrower sense of the term. If the bending is brought about by an irreversible growth-curvature, we label it a *mutation*, in contrast to reversible *variation movements* which are caused by changes in turgor and which occur at the nodes.

Orthogeotropism is the term employed if the reaction places the bending organ parallel to the direction of gravity: *positive* in those organs which are directed toward the center of the earth (main roots), *negative* in the case of perpendicularly upward-growing organs. Organs which in their normal position display any angle whatsoever toward the direction of gravity are described as being *plagiogeotropic*, and when the position is horizontal, that is, at an angle of 90° toward the immediate radius of the earth, a further distinction is made by employing the term *diageotropism* or *transverse geotropism*.

In contrast with curvatures of attached plants, the locomotive movements of free swimming organisms are designated as being *geotactic*. Scarcely anything worth mentioning has been added recently to our already meagre knowledge of this kind of botanical

phenomena and it may be omitted from further discussion in this paper.

In addition to the various stimuli which influence the direction of growth and which we have designated as geotropic in the narrower sense of the term, gravitation induces still other reactions which, by way of description, can be distinguished only with difficulty and which also are generally referred to in common botanical parlance as being geotropic (see Frank's definition). We speak of them as such, in the broadest sense of the term, though it might be more correct, perhaps, to designate them, in agreement with Zimmerman (66), as *geo-reactions*. Phenomena of this nature which are not genuinely tropic are those known as *tonic* and *morphotic*. The former include, among others, the effects of stimuli upon growth rate and intensity of reaction. *Geotonic* phenomena are involved when an invertedly fixed germinating shoot slows down in growth, perhaps ceasing entirely, or when a joint of grass, freed from a unilateral effect of gravitation by being on a clinostat, resumes growth after once having ceased to grow.

If gravitation induces a morphological differentiation between upper and lower surfaces, as in polarity and in the production of dorsiventral shoots and flowers (see Haeckel's (28) recent interesting contribution), we are then dealing with *geo-morphism*.

ORTHOGEOTROPISM, ANALYSIS OF THE REACTION-CHAIN

We may consider the question of details and of analysis of the geotropic reaction-chain best in connection with orthogeotropic reactions because they are the simpler; plagiotropic reactions, as we understand them today, are complex phenomena. The initial stage of the physical stimulus which we provide and the concluding stage of curvature which we ultimately observe readily lend themselves to experimentation and observation. Intermediate phases of the reaction-chain are more difficult of study; the most readily investigated of them is conduction of the stimulus, which can be observed directly if the foci of the stimulus and of the end-reaction are spatially separated. This has been known since Rothert's time for the seedlings of many grasses.

Reception (perception) of the stimulus. The initial physiological effect which the physical stimulus produces within the living cell seemed for a time to have been satisfactorily explained by the

statolith theory of Haberlandt and Němcě. According to this theory, any change in the direction of gravity induces a displacement of starch grains within the cell, the pressure (or pull) of which grains is supposed to exert a stimulus upon the neighboring protoplasm. In the 36 years since its formulation, a relatively great amount of material has been examined to test the accuracy of this theory. These tests have consisted, first of observations concerning the extent to which the occurrence of statolith starch and of geotropic sensibility are parallel, and secondly of attempts to control the statolith apparatus and, thereby, the geotropic sensitiveness.

Concerning a parallelism between the existence of statolith-starch and irritability, it is known to be frequent, but not without exceptions. This impression also results from Petschow's recent investigations on Bryophyta (42). It must be added, however, that no kind of statolith grain is known among fungi, many of which are very geotropic. Claims for this parallelism are limited, after all, to geotropism in its narrower sense. It is apparent from geomorphic reactions, however, that geo-stimuli are perceived also by many cells and tissues which exhibit no curvatures. Furthermore, there are certain plant parts which are generally regarded as unresponsive, such as fully developed tree trunks (22), but which, nevertheless, exhibit slow but very apparent bendings. Geotropic sensitivity is not at all limited, then, to the rapidly reacting apices of growing organisms, but the extent to which this parallelism applies in other cases of geo-sensibility has scarcely been studied.

Investigations upon the experimental production of influences by controlling the statolith apparatus have likewise led to no conclusive results. Zollikofer's experiments, which involved removal of the statolith starch grains, showed a decrease in geo-sensitivity and favored the theory. Studies by Buder, Zielinsky and Richter, involving a shifting of the statolith grains before the application of a geo-stimulus, were dubious, and von Uebisch (56) obtained results which are scarcely in agreement with the theory. A review by the present author (48) offers a résumé upon this subject.

Haines (29) has recently subjected the matter to a new investigation in which he exposed *Vicia*-roots (partly on a special apparatus—an impact clinostat) to lateral blows, rapid in one direction

and slow in the other. It is to be expected that such treatment would induce a change in the position of statolith grains and in the general contents of the cells, similar to effects secured by centrifuging. The observed curvatures took place laterally in the direction toward which the movements of statolith starch grains very likely occurred. Other geotropic theories also involve a change in the position of small particles of different weights, so that Haines' results can not be regarded as proof of the statolith theory.

Taking everything into consideration and in view of our present understanding, we can regard it as quite probable that there is a relation between starch and geotropism, but it is not necessary that the starch perform a statolithic function. Perhaps it plays a special rôle in the formation or activation of growth-hormones, concerning which more will be said later. Such a relationship is indicated by the work of Cholodny (17) who was able to show that in grass seeds the formation of growth-substance is associated with starch-bearing cells of the endosperm.

To-day a much discussed hypothesis is that of a geo-electrical perception of stimuli. This theory was set forth by Cholodny, Small, and Stoppel and placed upon an empirical basis by Brauner (12, 13). It is well known that electrical differences in potential can be demonstrated anywhere in living tissues and single cells; their causes lie in the membranes which induce an unequal distribution of positively and negatively charged ions, either within the walls themselves or in the surrounding medium. A differential absorption of ions or a differential permeability of the membranes toward these ions is involved. Membranes in this sense are not merely cell walls or parts of tissues, but within the living cells every plasma membrane becomes a carrier of a potential difference toward the cell sap or even toward parts of the protoplasm of different consistency. In short, all phase boundaries must be looked upon as being electrically charged, a condition which depends upon a definite distribution of negatively and positively charged ions. We know, furthermore, that very important life phenomena, such as imbibitional swelling and shrinking and the associated permeability of the plasma membrane, are influenced by the nature and quantity of such ions.

If an organ is exposed horizontally or at any other angle to the unilateral influence of gravity, the lower surface becomes positively

charged with a potential gradient of about 10 millivolts or more. This geo-electrical effect occurs also in dead organs and may be studied in models of parchment paper which have been saturated with an electrolytic solution. The explanation is not simple. Brauner was inclined at first to attribute it to a displacement of free moving and positively charged threads of liquid in the pores of the membrane; to-day, he and his student Amlong (2, 14) are inclined to believe that asymmetrical diffusion potentials develop at the bounding surfaces of the plant organ as a result of the unequal mobility of anions and cations.

Perceptible relationships exist between this geo-electric effect and geo-perception. If we place vertical organs in a transverse and sufficiently strong electrical field between two charged plates, changes in electrical charge will be brought about in the organs similar to those which are induced under the influence of gravity when the organs are horizontal. (Negative charge opposite the positively charged plate, positive opposite the negative plate). In this way electrically stimulated organs in Brauner and Bünning's investigations (15) bent according to the theory. The positively geotropic roots of *Vicia* bent toward the negative plate, and negatively geotropic coleoptiles of *Avena* bent toward the positive plate. Amlong (2) furnished confirmation of these results after Hartmann (30) had failed to reproduce them. Amlong was able to produce similar potential differences in another way, namely, by permitting electrolyte solutions of different concentrations to diffuse into the tissues of the opposite flanks of the root. Under these circumstances, too, the anticipated opposing curvatures of roots and shoots took place, independent of the chemical nature of the solution and dependent only upon the difference in the electrical potential. As in the case of geotropic stimulation, curvatures did not occur in decapitated shoots and roots. Koch (34) exposed roots and *Avena*-coleoptiles to an electrical field under water and both organs curved toward the positive pole, the coleoptile behaving as it did in air in the investigations of Brauner and Bünning, the roots otherwise. Since the coleoptiles are protected by their cuticle from the electrical stream in the water, the same polarization may be induced in them under water as in the air. But roots, permitting the electrical stream to pass through, are in the opposite position and within them the electrical gradient may be the same as in the surrounding water. In negatively geotropic organs, too, (seedlings of *Helianthus*

were used for technical reasons) there may be a deviation from the positive pole if the electrodes are inserted in the plant itself, an inductive, opposed polarization thereby being avoided.

In spite of all points in common, there are certain differences between the behavior of electrically and of geotropically stimulated plants. Electrical curvatures straighten out again after a few hours, even though the stimulus continues, while under similar circumstances geotropic stimuli become more pronounced. Further investigations are very desirable therefore, including positively geotropic stems and negatively geotropic roots, as well as with respect to ageotropic organs.

END-REACTION

Before considering the stages in the reaction-chain subsequent to perception, let us turn to the end-reaction which can be studied directly, and then take up the less accessible intermediate phases. The end-reaction consists of a curvature resulting from unequal growth of two flanks.¹ Only occasionally are variation movements involved, that is, reversible turgor movements which can be studied especially well in the nastic phenomena of articulations in the leaves of beans and of other legumes. Because these movements will probably be treated in a special article in this journal, it will not be necessary to enter into their mechanism (Summary in "Handwörterbuch der Naturwissenschaften").

The tropic growth reaction involves an increased elongation of the convex side while the concave side experiences reduced growth. Apart from new increases in growth as may occur in developed grass nodes, the average growth rate of the reacting organ remains, as a rule, unchanged. The difference in elongation rate of the two sides is associated with unequal access of water, the convex side absorbing more, the concave side less, than in the unstimulated state. According to Ursprung's nomenclature, the suction-force (S_z) of the cells is the controlling factor in their uptake of water. This force depends upon two quantities, S_i , which represents the suction-force or osmotic value of the cell contents, and W , the pressure of the more or less distended cell wall which tends to counteract the expanding tendency of the cell contents. This relation is expressed by the following formula:

$$S_z = S_i - W.$$

¹ According to Wagner (58), the number of mitoses is increased on the side becoming convex during geotropic reaction.

[We may disregard any outside pressure of the surrounding tissue as well as any other counteracting forces apart from the pressure of the wall itself.]

S_z increases upon the side which becomes convex and diminishes upon the concave side. An increase of S_z may come about by either an increase of S_1 or a diminution of W . We now know that the osmotic value of the cell contents (S_1) is changed during variation movements while during growth curvatures the wall pressure declines. As in normal elongation growth, growth of the cell wall is the primary process in tropic growth curvatures and not an augmented osmotic pressure of the cell contents, as De Vries, Darwin and Sachs assumed. While stretching, the cell wall offers a reduced resistance to the tendency of the cell contents to take in water by osmosis, so that additional water can enter the cells. The same relation with an augmented W applies to the reduced elongation of the cells on the concave side.

So far as the mechanism of this stretching growth is concerned, there is a series of investigations concerning the influence of the growth-hormone upon this process. Since Went's paper (63) in this journal contains an excellent summary on the subject, it need only be added here that growth-substance apparently does not exert its influence directly upon the cell wall. As appears from the investigations of Söding (51) and Bonner (6), among others, the growth-substance affects first the protoplasm which penetrates and is closely interwoven with the growing cell wall. As early as 1886 Wiesner characterized the growing cell wall as a living structure permeated by the protoplasm. This idea holds true also for fungi; see Borrius (7).

The recent investigations of Strugger (53) are significant in connection with further inquiry into the nature of these changes in the protoplasm. They indicate that the viscosity of the protoplasm increases during elongation growth, as is shown by plasmolysis. The same increase in viscosity is exhibited by cells whose growth is hastened by geotropic stimulus. According to Strugger, this increase in viscosity is the result of a change in acidity, a particular degree of acidity, differing from the isoelectric point, being characteristic of the growing cells. As a matter of fact, growth and growth curvatures may be induced in the same sense through the

influence of acidity. It is uncertain whether the acidity influences the degree of swelling directly, as Strugger claims, or whether there is a relationship between acidity and the efficacy of the growth-hormone. Probably there are still other relations, for various chemical changes within the stimulated organs are known to occur. Such changes have been claimed by Czapek, Kraus, Schley and Phillips as occurring in connection with geotropism; more recent investigations of Warner (59) have shown that there is a greater quantity of reducing sugar on the lower side of geotropically stimulated shoots than on the upper side (see 40). In agreement with Strugger's determinations, Gundel (26) found an increased pH on the convex side of geotropically stimulated roots and shoots. Furthermore, he found greater catalase activity on the concave side and, finally, differences in the color action of the two sides toward methylin-blue. All these observations, however, obviously bear no direct relation to geotropic reaction, for they take place not until several hours after stimulation, while curvature of the organs begins much earlier. Recent investigations of Friedrich (25) likewise point in this direction.

INTERMEDIATE STAGES OF THE REACTION-CHAIN

That there must be a series of intermediate stages between perception and the end-reaction is especially evident in those parts of the plant where conduction of the stimulus takes place. In many organs of the root and stem the apex is particularly sensitive to stimulus, almost solely so in some cases, while the curvature develops either exclusively or most conspicuously in a basal portion. The stimulus, or more accurately expressed, one of the first reactions induced by it, then progresses through the organ from the point of inception. We know that the growth-hormone, made known by Went, may play an essential rôle in this conduction, being transferred from the apex to the region of elongation. In unstimulated growing portions of the plant the hormone moves toward the base equally on all sides; in tropically stimulated organs it is deflected along one side. This was first shown by Went (61) in connection with phototropism; with respect to geotropism we are indebted to the corresponding work of Dolk (21) on coleoptiles of *Avena* and maize. Dijkman later secured similar results with *Lupinus* (19). The stimulus does not alter the quantity but only the distribution of growth-substance

which has been produced. If the coleoptiles are severed and the upper and lower halves placed on agar in the customary manner, the former show a minus, the latter a plus with respect to growth-substance. This is also true of roots but, as we have learned particularly through the work of Cholodny (16), the growth-substance in these organs induces a reduction rather than a stimulation of growth. It is more difficult to demonstrate growth-substance in the roots but this may be achieved by the addition of dextrose to the agar (9).

Translocation of the growth-substance ensues during the first hour after stimulation, in which time the first bending also takes place. In the nodes of grasses where the stimulus induces resumption of growth, there is additional formation of growth-substance (47). Certain influences which disturb formation of the hormone, such as ethylin (36) or erythrosin (10), hinder the geo-reaction. We see, then, that all these facts are in agreement with the theory of Went and Cholodny. A very important study, however, from the Berlin Institute shows that isolated root-tips growing in artificial media are perfectly free from growth-hormones. Nevertheless, they produce good geotropical reactions.

The mechanism of transport of the growth-substance is not yet clear, though it has been studied intensively, particularly by Van der Wey (60). That diffusion plays no significant rôle is apparent from the rate of transport as well as from the facts that it is associated with respiration activity (4) and is hindered by narcotics. It has been demonstrated, furthermore, that the transport of growth-substance does not depend on the vessels but can occur through the parenchyma cells. Whether protoplasmic streaming plays a major part, as appears very likely from the investigations of Bottelier (8), is brought into question through the polarity of the transport. And the well known fact that transport of auxin occurs better toward the base than toward the apex (see 32) requires further explanation. Perhaps an explanation is to be found in Went's "Theory of Polarity" (62), according to which the base of an organ is generally electro-positive as compared with the apex which normally discharges the growth-substance of an acid nature toward the base. Such removal of the growth-substance might be expected in tropic stimulation if the lower surface possesses an electro-positive charge. These ideas are in general agreement with the observed facts concern-

ing geo-electric effects. There are, however, certain observations concerning differences in polarity which do not appear to conform to the theory of polarity, such as those of Ramshorn (45), so that the entire question of transport and removal, which today is the subject of many new publications, still remains open. Discussion of this topic suffers particularly from our limited knowledge respecting the mechanism involved in the transport of materials in general.

It may be stated in conclusion, as the only certain fact, that growth-substance is transported through the living protoplasm, just as the effect of the growth-substance is exerted on the growing cell wall. The details of this process are not yet well understood. Conversion of the geotropic reaction chain into a series of physical causes has not yet been achieved. Instead of saying that the unequal distribution of growth-substance induces geotropic curvature, we should say, more accurately, that the plant undergoes curvature in utilizing the growth-substance which is unequally distributed. *Unequal distribution of growth-substances is but one of the means employed by the plant while reacting.*

TONIC AND MORPHOTIC PHENOMENA

There is a series of observations concerning geotonic phenomena, particularly in connection with those of W. Zimmermann (65). This investigator has shown that roots which are rotated perpendicularly to the horizontal axis of a clinostat do not remain uninfluenced but exhibit definite curvatures in conformity with certain laws. If such a root is exposed to the stimulus of gravity in a horizontal position and then changed to an inverted vertical position, the tropic effect is strengthened. If, on the other hand, the root is placed in a normal vertical position after exposure to the stimulus, perception and reaction are then diminished. Therefore, roots rotated on the horizontal axis of a clinostat react by curvatures. The stimulus resulting from the horizontal position of the root is less when followed by the normal downward position than when followed by the inverted upward position of the root-tips. Curvature thus sets in in the sense of the first horizontal position. In general, the following law holds true: the force of gravity parallel to the axis of the organ furnishes a tonic stimulus which has a diminishing effect upon the reaction when the organism is in the normal position, and a stimulating influence when the latter is in

the inverted position. This longitudinal effect (*Längskraft*) acts also at acute angles, the more so the more the organ concerned approaches the vertical position. These observations are now employed in explaining various phenomena, for instance, the optimum positions for stimulation of ortho- and plagiogeotropic organs. According to the law of sines, the influence upon orthogeotropic organs should be strongest when the latter are exposed to the stimulus at an angle of 90° , that is, when they are in a horizontal position. This is true also of brief exposures to stimulation, as the minimum stimulation (*Reizschwalle*) requisite for production of a curvature. It is not true, however, as Czapek has already shown, in the case of prolonged stimulation, as when the organ is long maintained in the stimulating position. In the latter case, stimulation was strongest when at an angle of about 135° , i.e., as the organ approached an inverted position. The tonic stimulation of the elongating force is apparently more potent then.

There has been no paucity in attempts to express these relationships numerically. Von Ubisch (57) assumes that the diminishing effect upon the reaction, whose relative value may be represented by k , is proportional to the cosine of the angle of deviation (α); its value would be $k \cos \alpha$. Metzner (39) regards this value as further dependent upon the magnitude of the geotropic stimulus to be hindered. According to the law of sines, the latter becomes $g \sin \alpha$, so that the tonic hindering effect in every case is expressed by the formula $g \sin \alpha \cos \alpha$. If a geotropic stimulus is exerted at the angle α , then the magnitude of the curving impulse is expressed by the formula $g \sin \alpha$ minus the counteracting force as expressed by the formula $g \sin \alpha \cos \alpha$. Metzner's formula for orthogeotropism then reads

$$G = g \cdot t \cdot \sin \alpha - g \cdot t \cdot \sin \alpha \cdot k \cdot \cos \alpha$$

where G = the total bending impulse and t = the duration of stimulation. Employment of such formulae is very limited, for they consist of quantities which can hardly be united into a single formula. The tonic hindering constant k , for instance, becomes effective only after a period of time, while the tropic impulse becomes effective sooner. With brief stimulation, therefore, the impulse is only as follows: $G = g \cdot t \cdot \sin \alpha$, and in this case the optimum stimulating angle is 90° . Not until the stimulus has been exerted in a constrained position for several hours is the hindering effect at its maximum. In the case

of such a long stimulation, it appears that Metzner's formula is applicable, for its calculated optimum stimulating angle of 120° , not 135° , can be confirmed experimentally (1). It is apparent, however, from the polemics between von Ubsch (57) and Metzner (41) that the value of this formula at other angles is slight and that errors in application and calculation can hardly be avoided because the employment of mathematics in this case contributes to the difficulties already mentioned.

Moreover, we know far too little concerning the manner in which the geotonic factor expresses its influence. For example, effects similar to those secured through an inverted position are produced by rotation around the horizontal axis of a clinostat where rapid revolutions (55, 49) and also intermittent stimulation (27) likewise increase the irritability. Beyond this, as Reiss in particular has shown, the observations are of difficult interpretation. They admit of neither the statolith theory nor the growth-hormone theory, particularly since Pfaeltzer (43) has shown that transport, utilization and effectiveness of growth-substance bear no relation to the longitudinal tonic effect. As the author has already noted (48), this effect involves a very peculiar phenomenon which follows its own but as yet hardly understood laws. It might be well to study geotonic phenomena in connection with another tropism, e.g., phototropism, for only then can "tonus" and tropism be clearly separated.

Geomorphotic phenomena include those cases where gravity influences the polarity or dorsiventrality of plants. Here, in addition to the previously known observations of Pont (44) concerning reversal of polarity in pendulous twigs of *Salix babylonica* which root at their apical ends when they reach the surface of periodically dried-out rivers in Africa, we must add those of Fitting (24) on the rôle played by gravity, light and substratum in inducing dorsiventrality in gemmae of *Marchantia* and *Lunularia*, as well as those of Knapp (33) on polarizing the eggs of *Cystosira*. Special interest attaches to the somewhat older investigations of Haeckel (28), a student of Goebel, which show that the dorsiventrality of gladiolus flowers and of other members of the Iridaceae is induced by gravity. On the clinostat they produce flowers which are radially distributed (see 31).

PLAGIOGEOTROPISM AND RELATED PHENOMENA

Plagiogeotropic phenomena have recently been the subject of a series of intensive investigations, reports of which have been some-

what polemic in character. Since the author himself is engaged in this branch of investigation, it would be embarrassing for him to attempt to give an account of the subject which at the same time should be short, clear and objective. The reader will find various viewpoints in the works of Zimmermann (66, 67), Metzner (39), von Uebisch (57) and of the author himself (48). Critical comparison will show that the differences are based more upon interpretation and terminology than upon actual observations.

The plagiogeotropic natural position of obliquely or horizontally oriented organs is determined not by any single reaction but rather by the coordinating and opposing effects of several curvature factors. This was known even to the old physiologists and has been repeatedly substantiated since the investigations of Lundegårdh (37, 38). An obliquely upward-growing lateral twig, for instance, possesses the normal negative geotropism of orthogeotropic shoots, but in this case this quality is opposed by a counteracting tendency toward curvature which, after the negative geotropic effect has disappeared, may be demonstrated as "reversions" of the organ. It has been shown on a clinostat as well as by treatment with ethylene gas, especially by Crocker, Zimmermann and Hitchcock (18). This latter tendency toward curvature was designated by De Vries as epinasty. This means an autonomic tendency of the dorsiventral organ to curve, the upper or dorsal side becoming convex. This tendency normally is balanced by the counteracting negative geotropism. As we know today (46, 67), this conception is thoroughly substantiated in many cases but there frequently arises a complication through the fact that the epinastic tendency of the upper side to curve oftentimes is not fixed from the very beginning of development but rather that external causes contribute toward determining which side will become the dorsal side of an organ. As we have seen in the case of geomorphotic phenomena, gravity, too, in addition to other external stimuli, can influence the direction of dorsiventrality. By fastening a young shoot in an inverted position we can force its original ventral side to develop as the dorsal side. The epinasty of the new dorsal surface which then develops is oriented by gravity. In the last analysis gravity is the factor which causes the upper surface to become convex. We can designate such a curvature as positively geotropic as well as refer to it as geo-induced epinasty. The former is the conception which we find in the works of Lunde-

gårdh, Zimmermann and Metzner; the older authors, as Pfeffer, who clearly understood the essential nature of the matter, have placed greater emphasis upon the epinastic character involved. The present author also regards this conception as leading toward an easier understanding. The relationship of this "geotropism" lies, moreover, decidedly more on the side of epinasty, a name which, by the way, must be maintained in all cases where intrinsic factors and not external conditions are involved.

Furthermore, the term "positive geotropism" suggests the idea of the geotropism which we know in the case of roots; actually, however, this "positive geotropism" is distinguished by many details of its as yet scarcely studied reaction-chain, and of its tonic sensibility, and particularly by the greater slowness of the stimulating effect. Though it therefore appears simple and more seductive to regard plagiogeotropism in many cases as the antithesis of positive and negative geotropism, a great many complications are concealed by this apparent simplicity. This is very evident also from the interesting analyses of many individual cases by Zimmermann (67), though he employs the terminology here rejected.

Metzner attempted to extend his formula to both types of geotropism in plagiotropic organs, but we have already referred to some of the reasons which appear to cast doubt upon the advisability of combining such heterogeneous phenomena into a single mathematical formula. This is especially true in the study of geotropism of lateral roots, as is apparent from the work of von Uebisch and the review of Metzner (41). We can draw only one conclusion from the polemics of these authors, namely, that the behavior of lateral roots is not at all understood. In fact, the matter is particularly complicated because root systems represent very unstable correlative systems. Lateral roots lose their plagiogeotropism very easily, by a severing of the main root or by a deviation of the main root from its normal position and by a placing of the entire system on a clinostat; in short, every experimental investigation has results which it is very difficult to evaluate.

It is worth mentioning that lateral roots can become negatively geotropic through treatment with copper salts in very low concentrations (64). It is questionable, however, to what degree this observation permits the conclusion that the natural plagiogeotropic position is acquired through the counteracting forces of positive and

negative geotropism. We do know, however, that geotropism becomes negative under different conditions also in the case of main roots, e.g., when there is a deficiency of oxygen in the substratum.

Finally, we must mention the twining plants in whose movements there is also a geotropic component. It is well known that these movements consist of circumnutations, which the free apex of the plant exhibits in its search for a support, and of winding movements after finding the support. With certain interesting exceptions, both movements are always carried out in the same direction, characteristic of the species, toward the left or toward the right. According to Darwin, these movements, the result of a progression of accelerated growth around the flanks of the organ, appeared to be of an autonomic nature. Noll and Baranetzki assumed the existence of a contributing geotropic stimulus. According to them, gravity induces an increased growth of one lateral flank (lateral geotropism), which accounts for both the nutating and the winding movements. As Ulehla could show, lateral geotropism is actually present in many cases. After considerable controversy, it was shown (47) that both views are justified; an autonomic factor undoubtedly is present in the movements of the organ as it seeks a support, an accelerated elongation traveling progressively around the shoot. In this way each flank successively becomes the convex side and in this manner the apex acquires a regular circular movement. The growing regions of circumnutating shoots are known to be very long, and the focus of autonomic curvatures is situated principally at the base of the elongated region. If the apex of a shoot is held immovable, as when it finds a support which prevents further circumnutations, then a lateral geotropic reaction becomes apparent, concentrated principally near the apex. The narrow circles which the plant now describes about its support are brought about principally through lateral geotropism (for exceptions and details see the original literature). It is not unusual that two types of reaction mechanism should thus work together, for the same is true also of tendril-bearing plants where autonomic circumnutations and haptotropism work together. These ideas of the present author have recently been confirmed in all their essential points in an investigation by Koning (35) at the Utrecht institute.

GEOTORSIONS

In addition to the tropic curvatures displayed by plagiotropic organs, the latter may also exhibit torsions. As was shown par-

ticularly by Schwendener and Krabbe (54), these torsions are genuine geo- or phototropic reactions, a view which is well substantiated today. The existence of such twisting reactions is of great significance in the physiology of stimulation as a whole, for all theoretical explanations, such as the light growth theory of Blaauw and the modern theory of growth-substances, if exact, must be applicable to torsions too. Meanwhile, the mechanism of torsions has remained unexplained until today. We are forced to assume that in torsions the growth of the twisting tissue undergoes a lateral deflection (48).

A recent study of Staub (52) has presented the surprising conclusion that such torsions may not be growth movements but imbibition phenomena of cell membranes. If this is true we shall then have to forsake our former conceptions which have seemed so well founded. The arguments which are advanced along this line do not appear to us to be sufficient, however. In many cases an increase in length of the twisting organs could not be detected and torsions occurred also in decapitated organs without access to growth-substance or to water. Were we to conclude, however, that in these cases the movement is not dependent on growth, such a deduction could not be quite convincing, as every investigator will testify, because in torsions only very small amounts of growth might be sufficient.

It was further noted by Staub that isolated and lifeless central cylinders display similar torsions upon withdrawal of water, torsions which are definitely attributable to imbibitional phenomena. The supposition of the author that in such cases the same torsion mechanism is involved, as we have in active geo-tropic reactions, seems somewhat dangerous because we know that in the latter cases no structural peculiarities of dead tissues can be involved. In living reactions the twisting proceeds to the right or to the left, as determined by the direction of the stimulus. Often, by progress of twisting to the basis of a petiole, the already twisted portions come in an "over-twisted" position, where new geo-irritation occurs. Torsion from now on sets in in the opposite direction. This phenomenon, well known since Schwendener and Krabbe, hardly can be regarded as controlled by structural features.

Finally, the so called "rotations" are very significant to Staub. They occur in isolated stem fragments which are in a horizontal

position and free to move with the dorsal flank beneath or on the side. In this position the organ exhibits a rolling motion until the dorsal side is on top. Such movements are not entirely unknown in the older literature. Organs handled thus, usually show lateral curvatures of an epinastic nature and the associated shifting of the center of gravity must induce such rotations in suspended organs more or less free to move. The author also often noted these curvatures but believes that the rotations can take place also while the organ remains completely straight. In any event, the occurrence of such rolling motions must be subjected to careful observation, for we can hardly regard them as conceivable. Such motions could be explained only through a shifting of the center of gravity from its position on the central axis of the organ. It is difficult from the very outset to see how this can happen and before these doubts are satisfactorily clarified we should not regard the hitherto accepted ideas as to be abandoned.

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ROOT INTERACTIONS OF PLANTS

W. F. LOEHWING

State University of Iowa

"The roots of plants are the least known, least understood and least appreciated part of the plant."—Weaver and Bruner.

As one surveys the monumental literature dealing with crop production, the small fraction thereof devoted to roots and the absorptive processes seems entirely out of proportion to their importance. Though actually meagre, our knowledge of root structure and development (59, 404, 405) is, nevertheless, much more comprehensive than existing information about the physiological activities and functional interrelationships of roots, especially as these are influenced by other organisms. Though ecologists and soil scientists have long stressed the necessity of regarding the soil as a biological unit, of which the growing plant is only a part, it is but recently that we have acquired sufficient explicit information on root interaction and activity of the soil microflora in relation to crop production for it to be of practical significance in determining agricultural practice. Gratifying as the advance in our knowledge of root processes has been in late years, it is modest indeed as compared to what may well be expected, once current diversified but unrelated research becomes coordinated and its results integrated. Consciousness of the need for information along these lines has long existed, as evidenced by more than a century of such research, but the data have been more descriptive than experimental, due largely to the hitherto insuperable technical obstacles attending control of the numerous variable factors necessarily involved under field conditions. Modern scientific investigations have disclosed that the development and activity of the root are more largely conditioned by the metabolism of contiguous roots and soil organisms than heretofore realized. These important problems have of late, however, received considerable attention as a result of the existing world-wide interest in soil fertility and attempts to establish permanent systems of agriculture, activities which perhaps justify an attempt to survey the outstanding advances of recent years. The scattered contributions on root interaction are conveniently brought together under the headings of toxic and non-toxic secretions, the latter topic being subdivided for purposes of clarity.

TOXIC SECRETIONS

Toxic root secretions quite naturally resolve themselves into the injurious effects of a given species upon its contemporaries, on the one hand, and upon its successors, on the other. The older literature (275) on this subject has been reviewed by Reed (308), Livingston (214), Russell (312), Czapek (89), Achromeiko (3), Bedford and Pickering (28). It is desirable to scrutinize the major findings of this early work for purposes of historical continuity as well as for re-appraisal thereof in the light of more recent discoveries on root interaction (39a). The original investigations on root excreta resulted chiefly from numerous instances of "soil sickness" due to one-crop agriculture on heavy poorly drained soils and from injurious after-effects of one crop upon another in certain systems of rotation (223, 258, 259, 260, 294, 330). Especially troublesome in grain belts of the world was the progressive diminution in successive yields of wheat when grown continuously upon the same soil under apparently optimal conditions of tillage and fertilization. Equally serious were losses from undiversified root-crop production. During recent years increasing frequency of "soil fatigue" has been noted following production of legumes (3b, 96). Outstanding among instances of injurious after-effects of one crop upon another have been the effects of sorghums, especially when used as green manures (41, 79, 123, 411, 416). Many cases of severe injury from sorghum stubble to wheat and barley have been reported from Kansas and the Imperial Valley of California as well as from Africa and India. The deleterious effects of sorghums were known to be markedly accentuated when the stalks were plowed under with the stubble.

There are, in addition to such common instances of injurious after-effects, many cases of harmful interactions among adjacent crops, such as the well-known decline of fruit trees growing near grass, as reported by Hedrick (168), Bedford and Pickering (27, 289, 290). Less common but perhaps even more striking than the foregoing are the depressing effects of the roots of walnut trees upon adjacent crops such as fruit trees, corn, alfalfa, potatoes and tomatoes (242, 321). Also frequently reported as deleterious to either adjacent or succeeding crops have been corn, rye, buckwheat, thistles, squash, sesame and the fleshy rootcrops such as turnips, rutabagas, mangels and potatoes (149, 338). Among the crops

most frequently injured by the foregoing are fruit trees, grapes, onions, alfalfa, tomatoes, potatoes and sesame (86, 90, 156, 160, 276, 279, 284, 296).

Injury in these instances was usually characterized by various combinations of the following symptoms: slow growth of root systems, inadequate or deranged nutrient absorption, chlorosis, premature leaf abscission in trees, slow maturation, delay or failure of reproduction, and waxy color of fruit when formed (28, 214, 219). The similarity of the symptom complex as a whole as well as of its individual characteristics in all of these cases suggested that toxic root secretions were probably the common cause of the injury.

The economic importance of the problems led to the well-known chemical investigations of Livingston (214, 216) and Schreiner (322, 323, 325, 326, 328, 329) in America and by Pickering (289, 290), Bedford (28), Russell (311), Czapek (88), and Aberson (2) in Europe. The American researches on certain notably refractory soils resulted in the identification of coumarin, cinnamic acid and the pyridine derivatives, picoline and piperidine, as well as of other toxic organic compounds (122, 324). An aliphatic dihydroxystearic acid was also detected and found to be injurious to root growth (327). Livingston showed that some injurious substances occurring in acid soils were colloidal and often volatile. Colloids such as carbon black and tannins were capable of detoxinating them, presumably by adsorption (214, 285, 421). Schreiner and Skinner (329) found that the intake of the essential elements, phosphorus and potassium, was depressed by certain phenolic compounds in the soil. Other fertilizers, however, served to antidote the harmful effects of phenols on nutrient absorption.

Massey (242) and Cook (82) have reported injury to adjacent crops by the roots of walnut trees. Davis (94) ascribes the effect to a non-diffusible root toxin "juglone," a strongly reducing quinone derivative. Ammonia, alkaloidal compounds and many organic acids have, in addition, frequently been reported as toxic excreta (122, 282, 297, 298, 299). Commonly mentioned among the latter have been formic, acetic, malic, lactic and oxalic acids (26, 84, 116, 128, 129, 170, 200, 203, 243, 253, 288, 304), the residual effects of which presumably produced a soil reaction unfavorable to crops. The treeless condition of American prairies has

been ascribed to similar effects of native grasses (282a). Bacterial soil toxins have also been reported (140, 141).

Most of the earlier findings on the excretion of organic acids and toxins by roots have, in fact, been of an inconclusive character and the results have not been readily reproducible (146, 150, 205, 348). Recent researches have contraverted many of the original conclusions by indicating certain experimental influences, especially selective nutrient absorption and ionic exchange, which had escaped attention or had been incorrectly evaluated (26, 89, 143, 200, 265, 307). The work of Lyon and Wilson (235, 415), for example, indicates that many of the organic secretions reported actually were disintegrated and highly dispersed tissues which had been abraded from roots during their natural growth into the soil (66). Some investigators find no excretion of organic acids of normally growing roots (348, 349) but under conditions of oxygen deficiency in the soil, roots initiate anaerobic respiration which causes the elimination of organic acids, as reported by earlier workers. Russell (315) points out that Pickering's observations on fruit trees were probably not due to toxins but to the utilization of combined nitrogen by surrounding grasses, a conclusion suggested by the fact that Pickering's results can readily be reproduced by withholding nitrogen. Russell's interpretation derives considerable support from experiments by Howard (178, 179) which show that marked injury from grasses can be overcome by use of ammonium sulphate. Grasses commonly impede the ventilation of heavy soils and cause injury to other crops, due to the resulting oxygen shortage and accumulation of carbon dioxide. Lack of free nitrogen under grasses also prevents nitrification. The treeless condition of American prairies has been ascribed to similar effects of native grasses (282a).

It has also been conclusively demonstrated in recent years that the injurious after-effects of sorghum and similar organic fertilizers are not ascribable to inherently toxic excreta but to the extremely rapid increase of non-nitrifying bacteria induced by the copious supply of soluble carbohydrates contained in such green manures (79, 163, 179, 249, 336a, 416). The profuse growth of soil bacteria immediately resulting from the available sugars introduced by green manures appropriates most if not all of the soluble soil nitrogen (5, 6, 109, 115, 185, 336), creating a deficiency thereof for

higher plants until again liberated during subsequent bacterial disintegration. The injurious effect of the large quantities of carbohydrates in organic fertilizers is definitely transitory (46, 135, 163, 180, 240), disappearing completely as the carbonaceous residues are oxidized. Farrow (120) noted the inhibitory effect of abscised pine needles upon the spread of *Carex* rhizomes, which is probably another example of a temporarily injurious organic fertilizer. Oriental workers (182, 183) find the catalytic effect of manganese and chromium salts of value in oxidizing organic matter, thereby shortening the injurious phase.

In addition to creating a temporarily unfavorable nitrogen balance in the soil, sorghum and other green manures are known to induce destructive deflocculation of mineral soils by augmenting the concentration of soluble alkalis, especially sodium (41, 163). Increments in free sodium entail packing and diminution in permeability of the soil (21). The deep root systems of sorghums coupled with their high water requirement may also create a severe insufficiency of soil moisture for succeeding crops in regions of restricted rainfall (80). Fortunately, however, green manures and other organic fertilizers ultimately produce beneficial effects in arable soils due to the resultant increase in bacterial nitrification during succeeding seasons (103). Sugars favor nitrate accumulation in soil by stimulating *Azotobacter* development wherever sufficient calcium carbonate, phosphorus and other essential mineral nutrients are available (31, 180, 331). It is now known that injury from carbonaceous fertilizers can be averted during the immediately ensuing season if they are plowed under well in advance of the next crop so as to provide a period of several months for a restoration of the biotic and nutrient balance in the soil (411). When fall plowing is not feasible and seeding immediately follows spring plowing, high carbohydrate manures must be used sparingly. Gainey (130) suggests not to exceed two tons of sorghum per acre. Though not wholly immune to carbohydrate injury, succeeding crops of legumes suffer less than graminaceous species (80).

On the basis of extensive data from sixteen crops grown on different soils in various rotations over a period of thirty years, Hartwell and his successors at Rhode Island showed that lowered productivity of local soils was not ascribable to toxic secretions nor to depletion of nutrients by heavy feeding crops, but rather to the

combined effects of altered soil pH and texture (51, 85, 156, 157, 159, 160, 276, 277, 278). The Rhode Island data, conclusive as they seem, may, however, require further verification, as they do not evaluate the effect of pH variations in terms of the concentration of soil solutes. Aslander (19, 20) and Lundegardh (226) have, in this connection, shown that the optimum pH for the growth of any species varies inversely with the strength of the soil solution (336b). Hartwell's experiments do show definitely that the actual nutrient requirements for many crops are surprisingly low whenever a balanced condition exists among the essential mineral elements in the soil (160). Heavy alkali feeders, such as cabbage, mangel, rutabagas and buckwheat, leave the soil acid and especially harmful to onions, a crop notably sensitive to acidity, especially in the presence of aluminum. Acid soils readily activate aluminum to the point of toxicity. The Rhode Island workers also found that though lime and phosphorus usually antidoted acid injury, heavy dressings thereof induced chlorosis due to insufficiency of manganese (156).

The ultimate conclusion to be reached from the more critical experiments on root secretions is to the effect that under usual agricultural conditions, appreciable quantities of carbon dioxide are given off, but that this does not usually reach a sufficient concentration on well-drained soils to be of harm *per se* (66, 69, 150, 151, 152, 164, 202, 265, 358, 377, 379). On heavy, water-laden soils, inadequate gas exchange may induce injury by impairing root development, nutrient absorption and nitrification (179). In brief, many investigators (304, 322, 355) share the convictions of Russell (312) who sums up the present status of the problem of toxic excretions by saying that "there is no evidence of the presence of soluble toxins in normally aerated soils sufficiently supplied with plant foods and calcium carbonate. Toxins, including hydrogen ions, soluble aluminum, iron and manganese compounds and organic substances, may occur on sour soils, badly aerated. There is no evidence of plant excretions conferring toxic properties on the soil." The evidence for bacterio-toxins is also disputed (181).

Waksman (402a) states that numerous instances of soil sickness previously attributed to toxic excreta are actually due to an increase of useless and pathogenic microorganisms at the expense of beneficial forms (282).

NON-TOXIC ROOT EXCRETIONS

If recent developments are taken as a criterion, a matter of infinitely greater agronomic importance than toxic excretions, is the liberation of significant quantities of nutrient elements in the normal metabolism of roots. Recent critical researches suggest that the phenomenon is widespread and a vital factor in the production of crops as well as in determining the botanical composition of characteristic associations of the native flora. In view of the importance of information along these lines, the scantiness of existing data can be ascribed only to the profound experimental difficulties attending quantitative studies.

Achromeiko (3), in an ingenious and valuable contribution, emphasizes the contrasts between root activity in soil and solution cultures. His own work as well as that of others reviewed by him indicates that many plants which show salt excretion in aqueous cultures give no suggestion of this phenomenon when grown in normal soils (16, 241, 243, 244, 245, 251, 300, 307, 371, 372). The elimination of inorganic salts, especially phosphorus, from the roots of legumes and certain other crops grown in soil cultures seems to occur normally after flower formation (3, 34, 39, 184, 340, 378, 410). Barley and probably other cereals excrete nutrients in late seedling as well as during maturation phases (49a).

The ability of legume, buckwheat, mustard and other roots to dissolve and absorb certain highly insoluble phosphatic minerals, followed by subsequent excretion of appreciable amounts of the phosphorus so acquired, gives these plants an exceptionally important rôle as a source of this nutrient for less vigorous feeders which readily utilize such excreted elements (3, 106, 295, 388, 410). Excretion of salts (87, 142, 283, 293) and of hydrolytic, oxidizing and reducing enzymes (4, 40, 43, 57, 235, 252a, 257, 326, 418) have also been reported by other authors.

Studies by the author upon dioecious forms of *Spinacia*, *Rumex* and *Cannabis* indicate that one of the chief secondary sex characters of the staminate plants is the greater number and amount of nutrient elements excreted by their roots during the floral phase. Flowering male plants, when rejuvenated by exposure to a photoperiod re-initiating vegetative development, show a definite diminution in nutrient excretion. Where sex reversal or hermaphroditism are induced by traumatism and by alteration of nutrients, light in-

tensity, temperature and soil pH, roots of reversed male plants in sand culture tend to assume the excretory characteristics normally shown by female roots. Root excretions of stamineate spinach plants, when experimentally converted to hermaphrodites, closely approximate those of normally hermaphroditic varieties. These observations suggest that nutrient excretion by typically hermaphrodite species varies as pistillate assume ascendancy over stamineate processes during maturation.

Herke (170), confirming earlier work by Mazé (243) and Schulow (332), adduces evidence that roots secrete sugar and malic acid, both of which he deems important in normal mineral nutrition. To malic acid he ascribes the solubility of phosphorus which would otherwise be precipitated by iron and lime in the soil. To the secretion of sugar rather than to carbon dioxide is imputed an accelerating effect upon the rate of phosphorus intake. Similarly, the recent work of Solberg (340) implies acid secretion, especially in legumes, in such a manner as to favor nutrient absorption. Mazé (243) and Knudson (199) have reported sugar excretion by roots.

The fact that organic root secretions, such as carboxylic acids, are considered injurious to plants by some investigators but beneficial by others suggests that there is no sharp line of demarcation between harmful and useful exudates. The effect of a given exudate may vary with circumstances, much like that of carbonaceous fertilizers already described. Despite several authoritative statements to the contrary, it seems justifiable to infer from evidence cited in this section that many roots normally excrete both organic and inorganic substances. Whether the effect is favorable to crops or not may depend as much upon the amounts as upon the chemical character of the substances involved. Thus the beneficial effects of a given acid in dissolving mineral phosphates may be offset by disturbed permeability of roots if the concentration increases unduly. Toxicity of such compounds is then a matter of quantity rather than of inherent chemical composition. The threshold of toxicity conceivably fluctuates widely with variations in the edaphic environment, minimal concentrations being most apt to cause disturbances under unbalanced nutrient conditions.

Rumanian workers under the guidance of Deleano (95) have recently extended the elder Chodat's original work on nutrient excretions by roots in a series of epoch-making researches. They

point out the inadequacy of interpretations based on chemical analyses expressed as percentages of dry weight, and the inability of percentages to disclose even large fluctuations in the absolute amounts of mineral nutrients. By determining the actual weights, they find that 30% to 60% of the absolute amounts of mineral nutrients including combined nitrogen are normally translocated from the foliage into stems and roots of trees prior to leaf abscission. A considerable proportion of the inorganic salts, except calcium which is rather immobile, is excreted by roots after flowering or abscission. The reverse translocation of salts is independent of the normal dehydration preceding dormancy or senescence of tissues, as it is also independent of the evacuation of soluble carbohydrate and amino derivatives which occurs much later. Nutrient excretion by roots had been postulated but not demonstrated by both De Candolle and Liebig. In 1865 the latter stated that "eine Ausscheidung von Excrementen kann demnach nicht geleugnet werden, wiewohl es möglich ist dass sie nicht bie allen Pflanzen im gleichen Grade stattfindet." Only recently have experimental data conclusively demonstrating mineral excretion as a normal phenomenon in natural soils been supplied in the meticulous works of Deleano (95), Achromeiko (3), and others (49a, 66a, 283). Whither further studies on nutrient excreta by roots will lead is, of course, an open question but from present indications they promise to shed considerable light on the all too nebulous subject of root physiology and thereby guide us at last to a biological appreciation of the plant in its entirety.

Tree roots also tend to restrict the luxuriance of undergrowth by limiting the water supply in the surface layers (248, 262a, 403).

INTERPLANTED LEGUMES

Important recent studies of root secretions have dealt with the relationship of legumes to other crops. The beneficial effect of legumes in rotations and in mixed plantings has long been known and properly ascribed to their ability to fix nitrogen in root nodules. Knowledge of the processes by means of which legumes exert their favorable effects has until recent years been quite fragmentary (363), with the result that agronomic practices employing interplanted legumes and legumes in rotation have been somewhat empirical as well as occasionally unsuccessful. As a result of the gen-

eral impoverishment of nitrogen fertility of the world's best agricultural lands and the ever-increasing use of legumes, the research on legume secretions has assumed tremendous importance. It has been peculiarly productive not only through its improvement of our botanical knowledge but also in unifying various other phases of plant research, largely due to the fact that the border-line character of root nodule physiology has necessitated the cooperation of microbiologists, agronomists and physiologists. Perhaps as the direct result of this many-sided attack upon the problem, progress has been gratifying and the results to date suggest that contributions will soon be made which will be as epochal as the discovery of nitrogen fixation in root nodules by Hellriegel and Wilfarth just fifty years ago.

Perhaps no phase of legume production has attracted more agricultural attention of late than their admixture with forage grasses in seeded pastures, a practice which has gained considerable momentum in Scandinavia, the British Isles and America. It is interesting to note in this connection that many such mixed plantings approach the botanical composition of the original native prairies in respect to dominant species. In fact, there is some evidence that even weeds may be beneficial to crops to a certain extent (42, 56). While we have more evidence as to the value of legume-grass mixtures, the whole question of mixed plantings of crops seems eminently worthy of more study (17, 71, 91, 104). The economic considerations underlying the use of associated legumes in artificial pastures are manifold, but predominant has been the attempt to maintain the highest level of soil fertility.

Despite the increase in our knowledge of legumes and their widespread agricultural use as soil conservers, the nitrogen fertility of the soil generally constitutes the most important problem in world agriculture. Any system of permanent agriculture must provide for the restoration of the tremendous losses of nitrogen due to crop removal and leaching. Löhnis (217) has stressed the significance of this question in America. A few years ago agricultural statistics disclosed that there were 375,000,000 acres under cultivation in the United States, approximately ten per cent of which was in legumes. On the basis of available data, it was computed that natural processes of fixation restore on the average about sixty pounds of nitrogen per acre per year under legumes and ten pounds under non-

legumes, making an aggregate replenishment of 2,500,000 tons per year. Commercial fertilizers total only 200,000 tons of nitrogen. Since 4,500,000 to 5,500,000 tons of nitrogen are removed by crops and by erosion in the United States annually, this leaves a deficit of 2,000,000 to 3,000,000 tons, a fact which manifests itself in a steadily diminishing fertility under continued use of agricultural soils.

Though associated plantings of grains and legumes is an ancient practice in the Orient (198), the agricultural advantages thereof were only recently emphasized in Europe by the controlled experiments of Pilz (293) and La Flize (206), and in America by the Cornell (229, 230, 231, 232) and New Jersey (210, 211) Experiment Station workers. Though the results of these important contributions were slow to find their proper place in agricultural practice, the production of legumes has become increasingly important, both as a crop and a system of husbanding soil fertility (48, 107, 108, 119, 186, 193, 197, 211, 212, 384, 408).

A fairly comprehensive understanding of the scope and character of recent investigations on the nitrogen nutrition of plants and the nature of legume-grass interactions is most readily gained from a rapid survey of current practices in pasture management (22, 136, 137, 189, 190, 210, 218, 228, 313, 333, 341, 342, 385). Mixed crops are somewhat difficult to harvest, a fact which accounts for the much greater use of intercropping in pastures over other phases of agriculture. Yet it has certain advantages even in the production of grains (22, 46, 272, 293, 388) and forest crops (67, 68, 92, 144, 403).

The advantages of intercropped legumes, as reported by Johnstone-Wallace (189, 190) for pastures in New York, are typical of the results reported in investigations along these lines (271, 291, 292, 385). He shows, for instance, that addition of two pounds of wild white (Kentish) clover to twenty-four pounds of forage grass mixtures (*a*) maintains nitrogen fertility of the soil, (*b*) suppresses weeds when properly cropped or grazed, due to the procumbent habit of the clover, (*c*) increases available lime, potash and phosphorus by its greater solvent action and the ability of its deep root system to raise these nutrients to the superficial soil layers in which grain roots feed. (*d*) Soil tilth and texture are greatly improved by the combination of shallow grass and deep-rooted clover. (*e*)

Mixed stands of clover and grass maintain fertility by conserving moisture through reduction in run-off and consequent prevention of erosion. Moisture absorption is enhanced by a profound increase in earthworm burrows over and above the numbers occurring in pure stands of either grasses or clover. (f) Moisture conservation and permanence of stand are associated with moderation of soil temperatures, i.e., avoidance of both high and low extremes. Average maximum soil temperature was reduced eight degrees F. at a depth of one inch between July and October on mixed plats, with individual daily reductions as great as fifteen degrees F. Such intercropped pastures continued to grow actively during periods of high temperature when open swards of Kentucky Blue grass alone had ceased all growth and assumed a burned appearance. (g) There was a gain of 1626 pounds per acre in total crop on the basis of average dry weight and (h) an increase of 6.3% in protein content of the interplanted over the pure grass pastures. It must be noted, however, that different legume species vary considerably in the amount of nitrogen fixed (233). In addition to these observations may be noted those of Kaserer (196) who observed a much more intimate intermingling and closer contact among roots in mixed than in pure plantings, a condition closely correlated with improved yield of the former. Fergus (121) finds that the beneficial effects of intercropped legumes persist even in years when they have been temporarily driven out. Many other researches report similar results for both intercropped and rotated legumes (22, 93, 107, 108, 119, 138, 194, 210, 252, 254, 271, 276, 298, 317, 347, 375).

Important as the existing practice of legume intercropping proves to be economically, it is doubtful if the interrelationships of the common pasture grasses with legumes would have received the critical scientific study they have, had it not been for certain difficulties which arose. Conspicuous among these in the field has been the effect of ordinary nitrogenous fertilizers in suppressing the legume components of mixed pastures and in reducing the performance of livestock grazed upon legume-deficient grasslands. Nitrogen is frequently added incidentally in commercial fertilizers containing phosphorus and potash, both of which are quite beneficial. Where injury occurs the selective action of nitrogen upon the legumes is very striking. It must be pointed out, however, that

apparently contradictory but authentic reports of beneficial effects from nitrogenous supplements have also been reported. These apparent discrepancies concerning the value of nitrogenous fertilizers for mixed plantings were subsequently reconciled by experimental data dealing with differences in the nitrogen nutrition of grasses and legumes. Headden ascribes the beneficial effects of clover and alfalfa upon other crops in Colorado to their "sanifying" action upon the soil and the ability of the legume roots to dissolve potash from feldspathic minerals (164, 165, 167, 346).

AMINO ACID SECRETION

Recent contributions in the field of legume root secretions include those of Virtanen and his associates in Finland. These workers purport to show that legume nodules normally secrete appreciable amounts of lycine and aspartic acid into the surrounding medium in experimentally controlled cultures adequately aerated with atmospheres of free oxygen and nitrogen (387, 388, 392, 396, 398, 399, 400). Grasses and uninoculated legumes in pure cultures readily assimilated these same amino compounds (388, 391, 394, 395, 400). The Finnish collaborators not only have identified the nitrogenous compounds synthesized by root nodules as aspartic acid and lycine but have shown that in addition to the part thereof absorbed by the legume host, large amounts of these acids normally escape from the nodules into the medium (391, 392, 400). The experiments were quantitative. They believe to have demonstrated conclusively also the dependence of the fixation processes upon the available supply of free oxygen and nitrogen (397). British and American investigators have reported the secretion in the host plants of unidentified substances which initiate infection of roots by nodule bacteria (2, 124, 327, 343, 359, 360, 362, 364). Starkey (343) has shown the greater abundance of soil bacteria in the rhizosphere where evidently they thrive upon root secretions and deciduae. Solberg's recent work definitely implies acid secretion by legumes (340).

The cycle of events thus experimentally determined by Virtanen comprises a triple commensalism, viz.: of legume host with nodule bacteria and ultimately of these unified symbionts with the grass. Associated grasses are not merely passive beneficiaries but definite symbiotic participants in this relationship because of their ability to

remove residual nitrates directly from the soil. Nitrate absorption by cereals facilitates nitrogen fixation by the nodules of interplanted legumes. It is evident from these data that any procedure endeavoring to employ only the nitrogen content of the nodules or even of the entire host plant as an index of its fixation efficiency, as has often been done in the past, is wholly inadequate and misleading. Diffusion of amino nitrogen to the soil must also be determined if any comprehensive impression of the fixation processes is to be obtained (35).

According to Virtanen (388), nitrogen fixation is a reduction process utilizing approximately 57 grams of glucose per gram of hydrogen produced. Fixation consequently entails a severe carbohydrate drain upon the legume, an effect which correlated well with the known high photosynthetic efficiency of legume over non-legume foliage (7). Virtanen (398) and others (9, 352) also direct attention to the fact that, if their data are correct, plants absorb through their roots sufficient amounts of complex organic substances to be of profound importance in their normal carbon and nitrogen metabolism. It may be noted here that Schreiner and Reed (324) in 1908 had reported the ability of plants to absorb amino acids and to use them directly as nutrients. The significant implication herein, namely, that plants are not entirely dependent upon the atmosphere for their anabolic carbon, appears to presage a revision of existing concepts of carbohydrates and perhaps also of mineral metabolism (4, 11, 40, 135, 215). These findings suggest that the physiologist has too long overlooked the fundamental significance of the common laboratory technique of feeding plants carbohydrates directly. In addition to the absorption of carbon and nitrogen in the organic form, we now have experimental data which indicate that legumes, at least, are also capable of absorbing some of their essential "mineral nutrients" in the organic form (332). Though these results require verification before they can be safely applied as generalizations they will represent important advances if confirmed.

Virtanen and his associates (383, 389) also noted that if certain maximal proportions in the number of grass to legume plants were exceeded, the beneficial effects of intercropped legumes diminished and even disappeared, an observation previously made by Lemmermann (208). The response is due to a competition for soil oxygen

which in turn reduces nitrogen fixation by the nodules, *pari passu*, with the degree of insufficiency (397). Legume and certain other types of roots appear to have both a higher oxygen requirement and a lower carbon dioxide tolerance than grains (58, 59, 219, 220). Lemmermann (208) ascribes the unfavorable effects of competition upon legumes to their poorer feeding ability resulting from a relatively low rate of transpiration as compared to grains. Through their more rapid nutrient absorption, thickly planted grains improve their initial advantage and eventually swamp out the associated legumes (148).

Quantitative studies by Virtanen in mixed cultures showed that a single pea plant could furnish ample amino nitrogen for the optimal growth of itself and of one or two associated oat plants. Greater ratios led not only to an inadequacy of nodule secretions but eventually to complete suspension of fixation (389, 395). Nicol (272) states that a stable balance among the botanical components in mixed pastures reflects a symbiotic relationship, while diminishing proportions of legumes imply competition. Failure to remedy disproportionate ratios among the components of pasture swards by heavier legume seeding accentuates the adverse effect to the extent of virtual legume elimination, a matter of great practical importance in livestock production because the protein content and hence the nutritive value of the herbage are reduced (148, 373, 374, 375, 376).

An important function of legumes in relation to nitrogen fertility seems to be their ability to stimulate *Azotobacter* growth as well as rhizobial development in the rhizosphere, thereby supplementing symbiotic with non-symbiotic fixation (218, 227, 234, 343). Achromeiko (3) maintains that excretion of nutrients by legume roots favors not only bacteria in the rhizosphere but eventually also the inoculation of roots by rhizobia. The importance of this problem makes it well worthy of further study.

As already intimated, agronomists have for many years noted the adverse effects of liberal supplements of ammonia and nitrates upon the proportion and growth of legumes in mixed pastures (5, 24, 44, 45, 125, 133, 177, 191, 270, 292, 339, 362, 365). The highly selective effect of the injury upon the legume components of the mixture accentuates its conspicuousness. By way of explanation, Nicol (272, 274) and others (386) point out that though

nitrogenous manures may increase the total yield of a mixed grass-legume crop, no appreciable quantity of the added nitrogen is recovered (Table I).

TABLE I

RECOVERY OF NITROGEN IN THE FIRST YEAR FROM AMMONIUM SULPHATE ADDED
TO A FORAGE MIXTURE OF OATS, BARLEY, VETCHES AND PEAS WITH A
BASAL SOWING OF FIELD BEANS (272, RECALCULATED)

Nitrogen added, lbs. per A.	0.00	2.24	4.48
Dry weight yield, tons per A.	1.30	1.78	2.50
Crude protein in crop, per cent	11.70	9.60	8.60
Nitrogen in crop, lbs. per A.	47.04	49.28	49.28

It is also known that the presence of excess nitrogen interferes with invasion of legumes by rhizobial bacteria (364, 367). Even if nodules exist, under conditions of high nitrogen they become reduced in number and finally become inactive (366, 368). Failure of legumes to maintain themselves on high-nitrogen soils is ascribable not only to their failure to develop root nodules under such conditions but also to their inability to absorb fixed nitrogen as readily as do non-legumes (208). In other words, while non-nodulated legumes can absorb nitrogen directly, they do not perform the process as efficiently as grasses and hence legumes are displaced when in competition on highly fertilized soils. These observations explain the common practice of using ammonium salts to eliminate clover from golf greens and garden lawns (33). The high-nitrogen or "hot" fertilizers, such as guano and cottonseed cake, are apt to be injurious to crops due to the liberation of free ammonia by soil bacteria (272, 413) and the microbiotic fixation of nutrients (3a, 3b).

The most desirable pasture requires the maintenance of a balance among the constituents of interplanted associations, a matter of considerable difficulty due to the complexity of the dynamic factors ordinarily at work. Upon the differential influences of climate and the natural tendencies to succession (78, 318, 374) are superimposed the variable effects of grazing, seeding and fertilizer practice (102, 341, 376). Though a fairly stable balance may, and often is, achieved during favorable climatic conditions regardless of seeding rate, it is found that prolonged drought and high temperatures induce very unfavorable alterations in sward composition. Abundant precipitation following drought definitely favors blue grass (117), especially on high-nitrogen soils (339).

The effect of high-nitrogen adjuncts in suppressing legumes intercropped with grasses is a forcible illustration of the ease with which the definitely symbiotic interrelation of grass and legume can be upset and converted into an economically serious competition. These observations suggest the need for care in nitrogenous fertilization of the so-called nurse crop of oats in mixed stands with clover and alfalfa. Under such circumstances the oats are of little value to the legume and they become more vigorously competitive as the applications of nitrogen increase in amount. Nicol (272) summarizes current practice on this point (1, 44, 45, 137, 179) as follows: "based upon a concept of a population existing not in rivalry but in harmony, the soundest method of manuring mixed vegetation is to supply abundant phosphates and potash, with lime if necessary, and by thus sustaining and increasing the vigor of the leguminous component, to encourage that double association upon which the natural well-being of the population depends."

NODULE BACTERIA AND THE C/N RATIO

Both invasion of the legume and fixation of nitrogen by nodules of infected plants depend largely upon certain nutritional conditions, among which the internal balance of carbohydrates to proteins of the legume is of paramount importance (11, 124, 126, 131, 132, 176, 280, 301, 381). Inoculation occurs only if the internal ratio of carbohydrates to soluble nitrogen is not extreme. Under very favorable photosynthetic conditions, such as high light intensity and abundant carbon dioxide, carbohydrates accumulate rapidly, widen the C/N ratio and thus retard nodulation and nitrogen fixation. Even well-nodulated plants under conditions of intense photosynthesis become unable to fix nitrogen. Any procedure which tends to better the balance of nitrogen with photosynthesis favors inoculation and fixation. This fixation-lag can most readily be overcome by the addition of small initial amounts of nitrogen fertilizers (11, 52, 172, 414), especially if combined with temporary shading or reduced temperature to retard carbohydrate formation (281). Interrelationships such as these probably constitute the rationale underlying the practice of "shading" cacao trees in the tropics with the larger leguminous Madre de Cacao, as well as the shading of tea, rubber and coffee trees (103,

248, 350). The beneficial effects noted in these cases undoubtedly represent the combined effects of increased soil nitrogen, aeration, differential feeding and optimal carbohydrate-nitrogen balance resulting from adequate but not excessive illumination.

Though the C/N concept does not explain all problems of nitrogen nutrition, it has been of prime importance in reconciling many apparently contradictory responses, especially to carbonaceous and nitrogenous fertilizers. Wilson believes that the question as to whether or not free nitrogen can be assimilated in the presence of combined nitrogen reduces itself to the effect thereof on the C/N balance in the host. It is probable that free nitrogen will be fixed, even in the presence of excess combined nitrogen, provided other conditions (light, carbon dioxide supply) are favorable for the maintenance of a C/N ratio sufficiently wide to permit fixation. Wilson's work, like that of other Wisconsin investigators (124, 281, 414), emphasizes the fact that a given environmental factor has varying effects upon the individual phenomena of root invasion, nodule formation, nitrogen fixation and host development. Only when all these processes are coordinated does normal symbiosis occur. Thornton (361, 362, 363) notes that as the C/N ratio narrows under a restricted carbohydrate supply, as commonly occurs in old nodules, the host tissues are actually parasitized. True symbiosis exists only under conditions of readily available carbohydrates. The addition of carbonaceous compounds to the soil, such as sugars, alkaloids, glucosides, and even the reputed toxins, oxalic acid, phenol and coumarin, stimulate nodulation and fixation just as increased carbon dioxide pressures do in low-carbohydrate plants abundantly supplied with nitrogen (14, 416). From this observation it seems justifiable to assume that the effect in both cases is achieved through widening of the C/N ratio.

Despite the great advances in our knowledge of nitrogen fixation by legumes, we do not yet know exactly how nitrogen from the root nodules gets into the host tissues, a matter of vital importance to further progress (35, 272, 274). Nicol (274) suggests that excretion of amino nitrogen has not yet been incontrovertibly proven, but only its occurrence in the rhizosphere. Neither has non-symbiotic nitrogen fixation been definitely precluded in the recent investigations already cited. Though interplanted legumes are generally beneficial, there are many cases of injury and loss in

yield on record (23, 113, 117, 118, 225, 247, 261, 262, 263, 406, 408, 409) for which we have as yet wholly inadequate explanations. Nicol is of the opinion that a comprehensive series of rigorously controlled experiments is necessary to elucidate these problems and to verify indisputably many prevailing assumptions relative to nitrogen metabolism. He proposes the general scheme which such experiments would of necessity have to embody to produce conclusive results.

The fact that bacterial invasion and activity are to a great degree conditioned by the metabolic state of the host, is a matter of profound interest in pathology, especially in relation to disease immunity (47, 70, 237, 238). It has been observed that the form of individual nodule bacteria varies with the functional activity of the host (60, 61, 62, 63, 64, 173, 174, 201, 320), bacteroid types flourishing in the root cells during the floral phases. Tissue extracts from flowering legumes have been reported to exhibit a maximal antibody coefficient, due apparently to the abundance of precipitins and agglutinins at this stage (54, 58, 353, 422). Production of bacteroid forms is associated with the presence of carbohydrates, and probably induced by the latter. The appearance of bacteroids is marked by a sharp delimitation in area of invasion and a reduction in the number of tubercles formed (25, 70).

From a phytopathological viewpoint, legume and tubercle responses (110, 111, 369) so closely parallel those of host and parasite in known cases of infectious disease as to suggest that legume symbiosis is merely a highly specialized type of acquired immunity. The legume appears to control bacterial action within its tissue by the production of antibodies (70). Analogous interpretations have been made of the symbiotic rôle of mycorrhizal fungi (29, 30, 236, 238, 239, 302). In view of the ability of certain soil microorganisms to reduce or wholly offset the injurious effects of pathologic species (65, 81, 147, 175, 192, 195, 212, 213), it is possible that nitrogen bacteria may also reduce pathogenicity of disease organisms in this way, though conclusive evidence on this point is still lacking. A phenomenon related to the foregoing is the competition existing among different strains of nodule bacteria (399). The result in this case is usually injurious, as useless components of the microflora may depress the active rhizobial population (124), or an inefficient strain of nodule bacteria may inhibit

the entry of an efficient one into host roots (8, 363). Bacteriophage also exhibits a variable effect, the degree of interference with fixation varying with the strain of bacteria (15, 96, 207, 370). The diminution in yield, or the so-called "fatigue" developing after prolonged cultivation of legumes upon the same soil, seems to be associated with microbiotic nutrient fixation (3b), with impaired nodule formation and possibly with lytic action of bacteriophage. Its harmful effects can be overcome by the use of fresh soil inocula (96) and fertilizers (114). These interactions among the microfloral components of the soil not only complicate their effects upon higher plants but also obscure the physiology of the nodule bacteria themselves. Complex and as yet obscure interactions of the foregoing types undoubtedly underlie the occasional instances of injury from legumes in rotations and in mixed plantings (6, 105, 118, 166, 263). In fact, the beneficial or harmful effects of fertilizers and tillage are often indirectly due to alterations in the soil microflora. Though no systematic farm practices have as yet evolved from our knowledge of microbial antagonism and symbiosis (402, 402a) it seems safe to predict that controlled soil inoculations may soon permit us to combat disease and influence crop development much more directly than has been possible by other means.

INTERACTIONS WITH NON-SYMBIOTIC BACTERIA

In view of the extreme importance of nitrogen nutrition to the economy of crop plants and the importance of bacteria in connection therewith, it is difficult to understand why we have not yet had more thorough physiological study of the non-symbiotic nitrogen bacteria. This is an important field eminently worthy of intensive study because of the possibility of increasing the supply of assimilable nitrogen in the rhizosphere of graminaceous crops (382). Clarification of the conditions required for optimal non-symbiotic fixation would add an important link to the understanding of biotic transformations of nitrogen in the soil (97, 98, 99, 100). While we already have meager evidence that rice and legume crops stimulate non-symbiotic fixation by *Azotobacter* and *Clostridium* (227, 316, 334), unfortunately we do not yet know precisely which cultural practices favor the activity. Bucherer (49) purports to demonstrate a symbiotic relationship between *Azotobacter* and certain molds. There is also evidence that *Azotobacter* stimulates the

growth of rhizobia (124). Stephenson (345) states that nitrification favors the mineral nutrition of crop plants. But our knowledge of these and kindred relationships is too meager to be of much practical value. Though these facts suggest the desirability of artificial soil inoculation, the use of such inocula has not, however, always produced the expected benefits. We need additional information on these biotic interactions to assist us in the efficacious preparation and use of commercial bacterial inocula. Though many workers (124, 169) consider the use of artificial soil inoculation of great value on low-nitrogen and acid soils, de Rossi (98) finds that most bacterial inocula which have thus far appeared on the market are of variable composition and practically pure nostrums.

While it is known that the so-called non-symbiotic nitrogen bacteria establish facultative symbiotic relationships with certain soil algae and that some nitrogen fixation results from this relationship (13, 48, 134, 250, 331, 402), we do not know if the conditions inducing the symbiotic are compatible with cultural requirement of soils, nor do we know if the degree of fixation in this manner is quantitatively significant to agriculture. Huneke (179a) calls attention to the ability of *Anabaena* to induce fructification in *Azolla* when in symbiosis. A phenomenon of considerable importance is the rôle of algae in the aeration of rice paddy soils (103, 154). Methane and other hydrocarbons resulting from the fermentive decomposition of organic matter are ultimately converted by surface bacteria to carbon dioxide, which is in turn utilized in algal carbohydrate synthesis. The resulting photosynthetic oxygen dissolves in irrigation waters, thus stimulating the penetration and hence the feeding radius of rice roots as the aerated water percolates through the soil. This oxygen also favors the activity of non-symbiotic nitrogen accumulation in soils and the resulting fixed nitrogen is, of course, absorbed directly by rice roots.

In connection with recent work on the necessity of certain so-called minor elements for plant development, it seems that as yet inadequate attention has been paid to the manner in which these substances influence crop production through their indirect effects upon nitrogen bacteria. While it is difficult to state which of the heavy metals among the minor elements are essential nutrients and which merely catalysts in bacterial metabolism, only very minute amounts are required in either instance. Molybdenum and related

elements in amounts as low as two parts per million catalyze nitrogen fixation by *Azotobacter* (32, 36, 48, 53, 139, 331). The work of Allison and Hoover (12) suggests that the coenzyme R needed for rhizobia is probably a heavy metal (344).

Other complicating transformations of nitrogen in the soil which create experimental difficulties and interfere with a clear understanding of nitrogen nutrition are those of purely photocatalytic fixation in the soil and the effect of organic materials on *Azotobacter* activity. The observations of Dhar (101), Corbet (83), and de Rossi (100) on physico-chemical nitrogen fixation have been disputed by Winogradsky (417), Joshi and Biwas (192), but it nevertheless seems probable that some increments in soil nitrogen occur in this manner (180, 303, 354). Rothamsted workers (180) show that *Azotobacter* requires a source of energy, carbohydrates from plant residues being quickly and effectively utilized by it in fixation. It should also be noted that non-nodulated legumes in the genera *Cassia* and *Crotalaria* form fully as much amino nitrogen as nodulated species, a condition as yet inadequately understood (209).

MYCORRHIZA

Also neglected from a functional standpoint have been the mycorrhizal fungi, above all those possessing the ability to fix nitrogen (112, 188, 287, 305, 306, 335, 419, 420). Though mycorrhiza may in general be more important in forest than in field crop production, they are, nevertheless, involved in root development of enough crops to justify their investigation from this angle (127, 236, 239, 286). Mycorrhiza seem to favor absorption of nutrients both by decomposing unavailable organic residues and by increasing the solubility of minerals (407). Fungal invasion of roots, symbiosis and parasitism appear to be conditioned by certain metabolic states of host and fungus, much as in legume bacteria (187, 305), and mycorrhizal fixation of nitrogen is similarly impaired by the presence of excess soil nitrogen.

The increasing importance of tree crops and of reforestation as an integral part of national soil conservation programs readily justifies additional emphasis on mycorrhizal researches (161). There is considerable promise that intensive physiological study of these unique symbiotic fungi would disclose the means of harnessing them in the service of mankind through their nutritive value to

human crops in much the same manner as has been so successfully done through our knowledge of root nodule bacteria. Certainly the most critical evidence indicates that mycorrhiza are often of benefit to their hosts, the degree of assistance merely varying with conditions of the external and internal environments (305),

The existence of certain non-leguminous root nodule bearers (204, 310, 401, 402) provides opportunities for comparative physiological studies of legume with non-legume nitrogen fixation. The presence of nodules on the roots of alders gives them a place similar to that of locust trees as nitrogen conservers. In this connection it may be mentioned that abscised locust foliage is known to be higher in nitrogen than that of non-leguminous trees (68), a fact which permits nitrogen accumulation in surface layers and thereby favors the development of more shallow rooted species (144).

GROWTH-PROMOTING SUBSTANCES

It has long been known that organic fertilizers, especially green and barnyard manures, are superior to mineral salts in stimulating crop development and reproduction (155, 266, 267, 268, 380). Since it has been shown that plant growth can be stimulated by *b*-indolyl acetic acid (162, 390) absorbed through normal roots, it appears highly probable that such growth accelerators can also be absorbed from organic manures added to the soil (224). At present this seems the most plausible explanation of the stimulatory effects of small amounts of organic matter observed by Nath (266), Tyagny-Ryadno (390) and others (268, 269, 393). Finnish workers (393) report the stimulatory effects of sterile extracts of yeast as due to the presence of heteroauxin, which they believe is also produced by many soil micro-organisms. The important implication herein is, of course, that soil organisms in general may serve as a source of growth promoting substances such as vitamins and hormones (39, 356). Production of such compounds by *Azotobacter*, rhizobia and mycorrhiza would give them an accessory function almost as important as their better-known rôles in nitrogen nutrition (286, 357). In fact, Thimann (357) has recently suggested that rhizobial auxins induce root proliferation which eventually results in the formation of nodules. This idea harmonizes excellently with the known stimulative effects of growth substances and the high concentrations experimentally demonstrable in root nodules, but it

offers no explanation of the restriction of rhizobial invasion to legume roots. In view of the fact that accessory growth substances required by plants may not be identical with the vitamins needed by animals, Nicol (272) has suggested the term *phytamin* to designate the former category of compounds. The term has much to recommend it, even at the risk of duplication in nomenclature, at least until the precise identity of the plant stimulants is more definitely established (264).

The research on auxins and phytamins promises aid in clarifying hitherto controversial features of earlier work on the essential character and stimulatory rôle of auximones, yeast, bacterized peat and other sterile organic extracts upon the growth of higher plants (18, 37, 38, 54, 55, 72, 73, 74, 75, 77, 153, 171, 255, 319, 351). It now appears that some of the effects previously ascribed to unidentified auximones are in reality due to phytohormones, vitamins or other quasi-catalytic agents (246, 256, 269, 352, 388, 412). Yeast and other microfloral extracts are known to accelerate flowering and fruiting in much the same way that pure solutions of crystalline hormones are known to do (52). These extracts contain hormones and phytamins to which a large portion of the response is undoubtedly due (314, 337). Burgeff (50) finds that orchid seeds in association with dead fungi germinate as readily as with living mycorrhizal species. He finds a heat-stable fungal extract capable of replacing the normal fungus in inducing seed germination, from which he concludes that orchids have merely lost the ability to synthesize vitamins and are dependent on their fungi for the same. Magrou (239) demonstrates the dependence of root development of orchids upon fungal invasion.

In direct contrast to earlier views, recent conclusive evidence discloses that organic matter is not essential for the growth of *Lemna* in sterile culture. Recent research indicates that organic matter merely acts as a growth stimulant (18, 72, 73, 75, 77). Clark (76) prefers to retain the term auximone for the stimulative agent in this case because he believes the substances which accelerate the vegetative development of *Lemna* are not vitamins or hormones in the strict sense. The specific stimulatory effect of humic acid upon *Lemna* in the presence of bacteria appears ascribable to the iron that it contains and provides in a form readily available to bacteria. Iron so supplied stimulates the growth of *Azoto-*

bacter in unsterile cultures and thereby the production of nitrates needed for the growth of *Lemna* (54, 55, 74, 150). It has also been found that the traces of mineral elements present in the organic adjuvants commonly employed in tissue cultures greatly accelerate the growth of excised root tips (309).

CONCLUSIONS

From the foregoing discussion of root secretions and micro-organisms in relation to plant development, it is evident that the more important agronomic problems are concerned with the maintenance of an adequate and continuous supply of nitrogen. Most of the older data ascribing soil sickness and plant injury to toxic root excretions now have been re-interpreted as the results of disturbed nitrogen nutrition rather than direct injury by toxins. Any appraisal of the present status of the work on root interactions would be incomplete without reference to the fact that recent investigations seek to determine the character of reciprocally beneficial as well as injurious effects among crops. Mere recognition of the favorable effects which often occur, such as those of legumes upon associated grasses, promptly resulted in major contributions as their causes. The distinction between toxic and beneficial root secretions, though objective in a practical sense, is actually arbitrary. The effect, good or bad, of a given root exudate upon other plants varies with circumstances, only a few of which have as yet been identified. Whether the effects of carbon dioxide or of carboxylic acids, for example, are beneficial or deleterious to crops may depend as much upon the quantity secreted and the insolubility of mineral nutrients in the soil as upon the rate of acid decomposition and removal. Viewed in the light of modern studies, much of the older work on acid excretion by roots conclusively demonstrated the occurrence of this phenomenon but erred in ascribing injury thereto, especially in instances of low concentration.

With reference to the beneficial effects of interplanted legumes, the most significant of the recent findings attributes this response to their ability to stimulate non-symbiotic as well as symbiotic nitrogen fixation and, as a result of the latter, to maintain continuous secretion of amino acids which can be assimilated directly by associated crops. Though the data on the secretion of mineral and organic nutrients by roots are fragmentary, there is considerable

evidence that amino acids comprise only one of several equally important groups of nutrient exudates. It seems safe to predict the early production of conclusive evidence showing that the transition from the vegetative to the reproductive phase of plant development is correlated with a profound internal redistribution of both organic and mineral nutrients (16, 288a), some of which are normally given off in appreciable quantities by roots of woody and herbaceous crops. The difficulties of perfecting reliable methods and of obtaining decisive evidence concerning such secretions are obvious but the urgent need and critical value of the data will undoubtedly provide the necessary stimulus for the mastery of the technical problems involved. Encouragement may well be derived from the success of studies on the physiology of the soil microflora, the interactions of which are much more intricate than those due to non-toxic root excretions of higher plants.

Worthy of note are the numerous techniques developed for quantitative studies of plants in the field, a trend which has served to bridge the hitherto great gap between physiology and ecology. The mutual interest and cooperative participation of agronomists, microbiologists and biochemists in questions of root interaction have not only produced practical results otherwise unachievable but they have also served as cohesive influences among these branches of biology, much to the gain of all. There has also resulted an increasing tendency to study the plant as a whole instead of treating root and shoot as separate entities. It is obvious, however, that further factual research on root excretions and interactions is required for the complete integration of the physiology of the root with that of the shoot and for the proper evaluation of the many edaphic factors influencing plant development.

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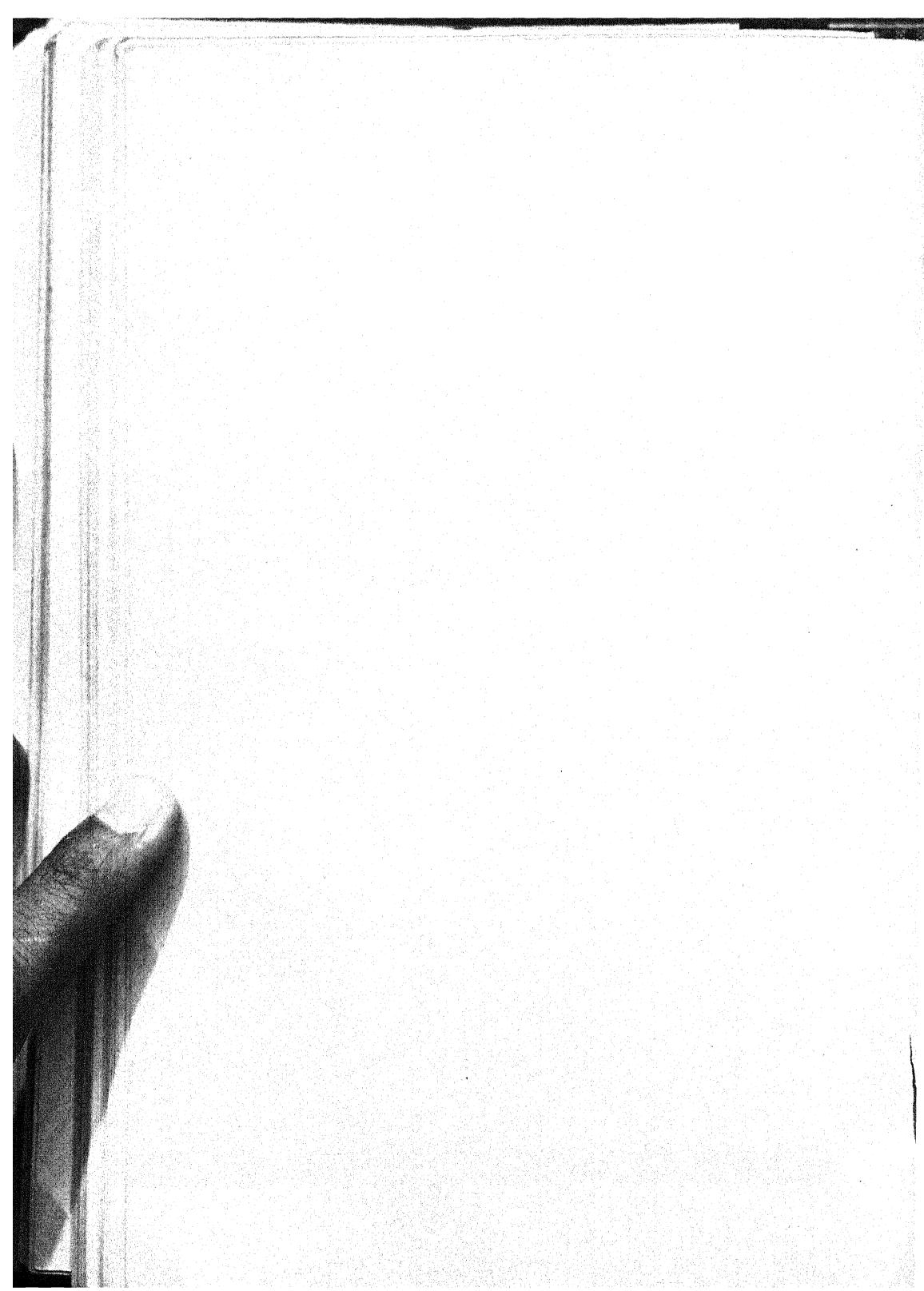
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THE BOTANICAL REVIEW

VOL. III

MAY, 1937

No. 5

IMPROVEMENTS IN PLANT CYTOLOGICAL TECHNIQUE

L. LA COUR

John Innes Horticultural Institution, England

Cytology, a rapidly developing science, has now reached a stage where technique is of more importance than ever for the successful interpretation of chromosome morphology and complex structures at meiosis. Every new advance in our knowledge depends on an advance in technique. Three innovations have been chiefly responsible for our present standards: the permanent and aceto-carmine smear methods and the gentian-violet staining technique. Since their inception, modifications and improvements of these methods, devised by various workers, have contributed in no small measure to their present perfection. For the improvement of our technique in the near future two recent acquisitions, the Feulgen stain and the dioxan treatment, are promising. The latter has several important properties and may be one of the most important reagents in microscopical technique.

FIXING TECHNIQUES

The Permanent Smear

The most rapid and, where it can be employed, the most satisfactory method of fixation is the permanent smear method which was first suggested by Taylor (57) for pollen mother-cells of plants. It has frequently been described in detail, together with modifications, and may be recommended for use wherever possible (35, 53, 38, 5). It may also be used for the first pollen grain division, as suggested by Darlington (16).

With suitable plants and animals the results obtained, especially in early stages of meiosis, cannot be excelled. The smeared cells come into direct contact with the fixing fluid, fixation being immediate. Preparations can be made in a few hours with a minimum of labour.

Smearing is done with the aid of a flat honed scalpel, or a second slide in which case both slides can be placed in the fixative. Small anthers, like those of *Tradescantia*, *Rhoeo* and *Petunia*, are crushed slightly with the slide or scalpel, the extruded cells being at the same time smeared over the slide. The aim should be to obtain an even smear in one movement, and it should not be necessary to draw the scalpel up and down the slide several times. Large juicy anthers, like those of *Tulipa* and *Lilium*, are best cut in two and the contents squeezed out onto the slide with the fingers before smearing. According to Belling, (5) it is necessary to dry anther segments with blotting paper prior to pressing out the cells; he suggests that the sap in the anther walls is deleterious to perfect fixation. In plants like *Paris*, *Trillium* and *Podophyllum*, where the cells hang together in strings, it is advantageous to cut the ends off the anthers and then press out the contents before smearing. A thin smear of Mayer's albumen often aids the sticking of small dry pollen mother-cells. This was found useful for pollen grains which are often too dry for smearing but which possess a wall so thick and persistent that it prevents success by any other method. Upcott and La Cour (59) found that a liberal quantity could be used without spoiling fixation or staining. Catcheside (13) finds the ordinary smear methods useless for pollen mother-cells of *Oenothera*. Success was obtained by taking a bud, stripped of sepals, and cutting across the tops of the anthers with a sharp scalpel. The bud was then squeezed from the base, the emerging cells being at the same time smeared on a slide.

Schedule I. Permanent Smears

FIX two hours. Longer does no harm. 15 mins. is sufficient for some purposes.

WASH in running water 5-10 mins.

BLEACH after osmotic fixatives in 1 part 20 vol. H_2O_2 + 3 parts 80% alcohol, 15-30 mins.

RINSE in water before staining.

Bleaching is not always necessary in the case of pollen grains (16).

Iron Aceto-carmine Smears

Belling's iron aceto-carmine method (6), which consists in teasing the pollen mother-cells out with nickel needles into a drop of aceto-carmine¹, besides being useful for determining stages of division, enables excellent slides of most phases in pollen mother-cells

¹The formula for this solution is given later.

and pollen grains to be obtained with little effort. The following suggestions have been made for improving the quality of preparations, especially in the case of small chromosomes, which are often more difficult to stain:

1. Barrett (3) finds that the use of haematoxylin in acetic acid gives intense staining. The method does not appear to be very popular. With many plants the cytoplasm is inclined to stain.
2. McClintock (45) has invented an improved method for maize which is of wide application. It consists in fixing the anthers for 12 to 24 hours in alcohol-acetic acid before teasing the cells out in aceto-carmine. If the material is not to be stained immediately, it should be stored in 70% alcohol.
3. Gentle heating over a spirit flame increases the contrast between chromosomes and cytoplasm, but the solution must not be allowed to boil. Heating also flattens the cells and spreads the chromosomes. As the degree of spreading is due to the pressure of the cover-slip, it is controlled somewhat by the amount of aceto-carmine used.
4. The edges of the cover-glass should be sealed with vaseline, rubber solution, or the sealing medium suggested by McClintock (39).

Permanent Aceto-carmine Smears

Preparations in aceto-carmine are generally improved after keeping for a few days, but after a time the chromosomes show some disintegration. The life of preparations is lengthened by keeping in a cool place, or on ice, as proposed by Belling.

Methods for making aceto-carmine preparations permanent have been described by McClintock (44), Buck (9) and Steere (56). The last method is suitable only for plants that can be smeared, but is the quickest means known of making permanent smears. The method of McClintock has been used by many workers with great success. Beginners sometimes experience difficulty in avoiding loss of cells. Success should be obtained if the following rules are observed:

1. Slides and cover-glasses must be scrupulously clean to avoid air bubbles.
2. All anther debris must be removed from the slide after teasing out the pollen mother-cells. In the removal of debris many cells

may be lost; where material is scarce and valuable, a second slide may be made from the fragments.

3. No more aceto-carmine should be used than will permit of the cover-glass being placed in position without air bubbles. This is important if the pachytene threads are to be flattened.

4. The amount of intermittent heating required must be determined by practice and will vary for different stages of division and in different plants. Early meiotic stages seem to require more heating than later stages.

Schedule 2. Permanent aceto-carmine smears

1. FIX the flowers, or in the case of cereals the whole spike, in 3 parts absolute alcohol to 1 part acetic acid for about 24 hours (in any case less than 48 hours). Store in 70% alcohol.
2. DISSECT out the anthers and crush them in a drop of aceto-carmine on a slide. The flat end of an aluminium needleholder is a good instrument for this purpose.
3. Remove all visible debris, anther walls, etc., from the slide before placing the cover-slip in position.
4. HEAT the slide gently over a spirit flame. This dries the excess aceto-carmine, making a rim round the edge of the cover-slip, so that when this has to be replaced, it can be replaced in its original position.

Repeat the heating at intervals (4 to 6 times) but never make the slide so hot that it will burn the hand.

5. Place the slide cover-glass downwards in a ridged dish containing equal parts of xylol, absolute alcohol and glacial acetic acid. After 5-10 mins. the cover-glass will float free and sink to the bottom of the dish. Never hurry this process by loosening the cover-slip with a needle, as any but the gentlest movement at this stage will detach most of the material from the slide. After separation let the slide and cover-glass remain an additional five minutes in the mixture.
6. Place the slide in two changes of equal parts of xylol and absolute alcohol, 5-10 mins. in each.
7. Remove the slide, place a drop of Canada balsam on it. Remove the cover-slip and put it back on the slide as far as possible in the original position and right side up. This should be done quickly to avoid the absorption of moisture by the medium and consequent clouding. If cloudiness appears, place the slide on a hot plate for a short time and it will clear.

Root-tip Smears

Aceto-carmine has been used with variable results for chromosome counts in root tips by Heitz (29) and more recently by Whitaker (63), and as a permanent method by Warmke (66). Heitz

(30) makes use of the Feulgen stain. This stain at its best² is superior to carmine in these root smear techniques. The disadvantage of root smears is the difficulty of finding a sufficient number of plates in polar view. The techniques referred to are most suited to plants having low chromosome numbers where they are useful in determining the direction of coiling in chromatids, the position of centromeres and trabants, or for rapid chromosome counts in a large number of plants known to be either diploid or tetraploid.

A rapid combined fixing and staining schedule is recommended by Backman (1) for material to be sectioned. The replacement of picric acid in Bouin's fluid by anthraquinone makes possible the addition of alizarin red for staining. The same fixation can be applied for smear preparations. Satisfactory preparations of the root tips of many plants can be made by combining this method with a dioxan paraffin schedule, using the smear technique as for pollen mother-cells. The writer's results have been variable.

Fixation for material to be embedded

If whole organs are to be fixed, as is usually necessary for root tips and small anthers, it is advisable to pump immediately after fixation to facilitate penetration. Fixatives containing saponin are to be recommended, for this reagent reduces the surface tension so that the material sinks more readily. This is particularly valuable with small anthers which often possess a waxy covering. If critical fixations are required, the anthers should be dissected out of the flower and fixed separately (17). This is a tedious process but well worth the trouble. In most cases pre-fixing with Carnoy is not necessary if the anthers are dissected out, but in some plants, e.g., *Primula*, the results are superior if the anthers are first dipped in Carnoy's solution for a few seconds and then transferred to the fixative (Darlington, 17, and unpublished). Material that has been treated with Carnoy previous to fixation should never be pumped, as this drives the alcohol and chloroform into the tissues and produces an inferior fixation before the osmic-chromic mixture can penetrate.

FIXING FLUIDS

The most satisfactory fixing reagents for plants and animals are osmic acid, chromic acid and acetic acid. These are usually used together in varying combinations.

² See below, Sect. 4.

Several workers have suggested new compounds as substitutes. For example, ruthenium tetroxide (12), salicylic acid and orthophosphoric acid (14) and uranic acid have been used instead of osmic acid in Flemming's solution (13) and chromic fixation in alcoholic media (61). Lewitsky (41, 43) has carried out a systematic investigation on the question of fixation. Most of his work has been on different concentrations and combinations of chromic acid, acetic acid and formalin. He has also suggested the possibilities of substances other than chromic acid that can be combined with formalin and claims to have obtained good results with some of these mixtures. My own tests of these fluids on *Tulipa* root tips yielded poor results. Poor results also obtained with the fixatives of Cohen which I tried on various plants. In the use of uranic acid I experienced the difficulties mentioned by Catcheside (13); the results are often good but variable. The fixation with ruthenium tetroxide also varies a good deal. Navashin's fluids are to be recommended where economy is of importance. The fixatives I suggest below are less expensive than the Flemming formulae and often give superior results. It should be noted that fixatives which swell the chromosomes slightly, also contract them lengthwise and, therefore, make for ease in counting; but, on the other hand, they often obscure points of attachment and secondary constrictions. This is the case with some Navashin fluids, and if they must be used, a type should be found by experiment to suit a particular plant. Lewitsky (42) has shown the different effects of these formulae with different plant material. Furthermore, he is of the opinion that fixatives used in chondriosome work usually also give good fixation images of the chromosomes.

Some of the better known fixatives are given below, with an indication by the present writer as to which in his opinion are the best for specific purposes. A small amount of saponin may be added with advantage to all aqueous fixatives, as it reduces the surface tension.

Types of Fixative

1. *For root tips or pollen mother-cells*, especially those with small chromosomes.
Navashin type (chromic, formalin, acetic). Karpechenko (34), Belling (5), Langlet (40), Lewitsky (42), Randolph (51), designated "Craf."
2. *For general use: smears of pollen mother-cells or pollen grains*, Flemming-Benda type. (Chromic, osmic, acetic).

Modifications by Taylor (57), Darlington (16, 17), La Cour (38) containing additional reagents, and designated 2 B, 2 BE, 2 BD. Ethyl alcohol 96% or absolute has been suggested by Yasui (64) for smears.

3. Root tips. 2 BE, 2 BD, "Craf."

4. Anthers (pieces if large). Medium Flemming; "Craf."

Anther contents: medium Flemming, 2 BE.

A method has been suggested by Mather (44) for some species of *Lilium* that have very dry anthers. After cutting off one end, the anther is pressed between finger and thumb; the pollen mother-cells come out in strings and are placed immediately in the fixing fluid. The cells coagulate and hang together in the fixative sufficiently to allow them to be passed through the paraffin schedule for microtoming. The results obtained are excellent.

5. Bouin-Allen. (Picric, chromic, formalin, urea).

6. Whole buds, unexposed anthers (Kihara 36).

A Carnoy fluid 6:3:1 should be used for one minute or more to secure surface penetration. The buds should then be rapidly rinsed in water before fixation is continued in one of the following: Medium Flemming, 2 BE, "Craf."

Formulae recommended for Fixatives

	Strength of solution
<i>Medium Flemming</i>	
30 c.c. chromic acid	1%
10 c.c. osmic acid	2%
25 c.c. acetic acid	5%
<i>Benda (low acetic)</i>	
30 c.c. chromic acid	1%
10 c.c. osmic acid	2%
5 c.c. acetic acid	5%
<i>2 BE</i>	
90 c.c. chromic acid	1%
1 gm. potass. bichromate	
0.05 gm. saponin	
10 c.c. acetic acid	5%
15 c.c. osmic acid	2%
45 c.c. distilled water	
<i>2 BD</i>	
100 c.c. chromic acid	1%
100 c.c. potass. bichromate	1%
0.1 gm. saponin	
30 c.c. osmic acid	2%
30 c.c. acetic acid	5%
<i>Craf</i>	
Solution A: 1 gm. chromic acid	
7 c.c. acetic acid	
92 c.c. distilled water	

Solution B: 30 c.c. formalin
70 c.c. distilled water

Mix A and B in equal parts just before using.

Belling

Solution A: 5 gms. chromic acid
50 c.c. acetic acid
320 c.c. water

Solution B: 200 c.c. formalin (or 100 c.c.)
175 c.c. water (or 275 c.c.)

Mix A and B in equal parts just before using.

Aceto-carmine (after Belling)

Iron aceto-carmine is prepared by mixing 45 volumes of acetic acid with 55 volumes of distilled water, heating to boiling and adding about 1 gm. of carmine to each 200 c.c. On cooling, the mixture is filtered and a drop or two of ferric salt solution can be added (preferably the acetate). Too much iron precipitates the carmine.

Sealing Medium

Equal parts gum mastic and paraffin wax. Heated and well mixed. To be applied with a heated wire.

3. DEHYDRATION AND INFILTRATION

Of all the branches of cytological technique, dehydration has been the most improved. The slow tedious methods in common use a few years ago have been considerably shortened (38). Where tissues used to be washed in running water for 24 hours, they are now washed only for an hour or two, and by some workers the washing is entirely eliminated. Randolph, in his new method (51), takes root tips direct from the fixative into 75% alcohol. A similar technique has been used by Upcott and La Cour (59) with good results.

It has been the common practice for two agents to be used in the process of dehydration, alcohol and xylol or alcohol and chloroform being the two usually favoured. The latter combination gives the better results. With the new methods it is possible to rely on one fluid to do the work of two. To be successful a reagent must be miscible with water, it must be a solvent of paraffin wax and must preserve tissues with a minimum of shrinkage and hardening. The two most notable discoveries are normal-butyl-alcohol and dioxan (diethylene oxide). The former was originally recommended by Zirkle (65) for woody tissues. It can be used successfully in general technique although, as mentioned by the present writer (38), infiltration is difficult for some material. More re-

cently it has been used by Randolph (51) in his new method instead of alcohol and xylol. He claims that this schedule gives results superior to those obtained by the present writer's method (38).

The possibilities of dioxan were first suggested by Graupner and Weissberger (26, 27). This solvent has also been used with some success by Johansen (28), Backman (1), Baird (2) (applied to animal tissues), McClung (46) and by McWhorter and Weier (47), who discuss its uses in general botanical technique. These workers find little shrinkage and hardening of tissues after dioxan and claim results superior to those obtained by other methods. Dioxan is more expensive than alcohol, though the schedule proposed by Graupner and Weissberger is an economical one. This method, however, is not entirely satisfactory, for it is difficult to avoid shrinkage of tissues during infiltration of wax. For the best preservation a slower schedule is to be preferred.

The writer has used alcohol-chloroform for a number of years on anthers, root tips and flower buds, with satisfactory results. Very little shrinkage occurs after such a schedule; buds may be hardened more than with normal-butyl-alcohol or dioxan, but there is no difficulty in securing infiltration of the wax. Where, however, the saving of time has to be considered, dioxan is recommended as slightly preferable to normal-butyl-alcohol. A revised alcohol-chloroform schedule is given below and a tentative dioxan method for root tips. The problem of infiltration is being thoroughly investigated at Merton with a view to improving the results with dioxan.

Other dehydrants reported to be successful are tertiary-butyl-alcohol, which Johansen (33) prefers to normal-butyl-alcohol; isopropyl-alcohol (8); and methylal-paraffin oil (22). La Cour and Rutland are investigating the possibilities of ethylene-glycol-monoethyl-ether, which can be used in the place of ethyl alcohol in the old schedule. Like dioxan this fluid is a solvent of balsam and could, therefore, perhaps be used to simplify some of the other techniques.

Schedule 3. *A revised alcohol-chloroform method*

FIX 12-24 hours.

WASH in 2 or 3 quick changes of water in the fixing tube.

DEHYDRATE in 50% alcohol	3 hours ³
70% "	overnight
80% "	3 hours
95% "	3 hours
absolute "	overnight
abs. alc. 3 parts, chloroform 1 part ...	2 hours
" 2 " 2 parts ...	2 "
" 1 part " 3 " ...	2 "

Then in pure chloroform with a piece of wax.

EMBEDDING. The tubes should now be placed on the oven top or hot plate, temperature approx. 30° C. More wax must be added every day. Two days are generally sufficient for complete filtration of the wax. Infiltration and evaporation of the chloroform are finally completed inside the oven with the cork removed from the fixing tube. Evaporation of the chloroform proceeds more quickly if the contents of the fixing tube are transferred to a small watch glass on being put into the oven. Four hours are usually sufficient.

A considerable amount of time may be saved in cutting and staining if several root tips are embedded close together so that they may be cut together. It is often difficult to arrange the material in the hot wax, owing to the rapid cooling when the material sticks to the needle. There is then danger of damaging the material by overheating the needle in a flame. Fabergé and La Cour (23) have devised an electrically heated needle which overcomes these difficulties. It remains at a constant temperature and, therefore, neither burns the material nor allows it to stick by cooling.

Schedule 4. *A tentative dioxan method for root tips*

FIX 12-24 hours.

WASH in 2 or 3 quick changes of water in the fixing tube.

DEHYDRATE in 1 part dioxan in 3 parts water	2 hours
2 parts " 2 " "	2 "
3 " 1 part "	2 "

Then dioxan overnight.

EMBEDDING. Place in the oven for infiltration, gradually adding wax of low melting point. Four hours is usually sufficient. Transfer to pure wax, leave for two hours, then embed.

4. STAINS FOR CHROMOSOMES

The principal stains used are gentian-violet (49) and haematoxylin; the former is to be preferred for critical observations. The staining procedure is simple and rapid (see schedule). Sections 4 to 40 μ . in thickness can be stained with ease, the chromosomes being well defined and the cytoplasm clear. This is impor-

³ After Navashin fixation tissues can usually be taken directly from the fixative into 70% alcohol.

tant when studying plants with large chromosomes, such as *Lilium* or *Fritillaria*. Not a few of the erroneous chromosome counts and interpretations of past years can be attributed to the use of haematoxylin; differentiation in thick sections being a difficult task, sections were cut too thin with the result that many of the plates were incomplete. The only disadvantage of gentian-violet is that the stain fades, its permanence varying with the source and the type of dye used. I have known slides ten years old to retain their stain where others stained with dye of different origin faded in a few months, and I have also noticed that the red types of gentian-violet are more permanent than the blue. Staining is often more difficult with the former, the cytoplasm being sometimes tinged red. In the absence of complete standardization in this country, staining problems and difficulties are more acute than is the case in America. It is possible that these reddish violets are actually methyl-violets. For a full discussion of the matter the reader is referred to the work of Conn (15) and Peterson, Conn and Melin (50). They suggest that the name "gentian-violet" is meaningless and should be abandoned, and distributors of stains induced to specify methyl- or crystal-violet, as the case may be.

Smith (55) claims to have a method of making gentian-violet permanent, the technique employed involving the use of picric acid. Slides prepared by this method certainly give intense, rather black, staining, but it is doubtful if the stain is retained appreciably longer than in normal preparations. Chromosomes of *Tradescantia* stained by this method, using a blue type of gentian-violet mentioned above, retain the stain for eight months as against the usual (approximately) six months. The picric acid which precipitates the dye possibly reacts differently with various types.

With certain plants, particularly those with small chromosomes, and after certain fixatives, it is sometimes difficult to obtain a satisfactory stain with gentian-violet. In such cases the following modifications of the ordinary staining schedule are to be recommended: slides are differentiated in a 1% aqueous solution of chromic acid after the iodine treatment (38), or in a saturated aqueous solution of picric acid (32). The principle involved is much the same in the two methods. The stain is precipitated in the chromosomes, allowing of its removal from the cytoplasm more quickly than from the chromosomes.

A method of staining large chromosomes, such as occur in liliaceous plants, is described by Upcott and La Cour (59). The chromosomes of these plants at the metaphase stage of meiosis stain too quickly in gentian-violet of normal strength. We recommend a 1% solution, as this weak concentration gives sharp staining and avoids long differentiation, which, after this stain, is inherently bad.

If the need for permanence demands the use of haematoxylin, we recommend the method of Tuan (58), *viz.*, the use of picric acid rather than iron-alum for de-staining. The writer has used a short staining schedule including picric acid with some success. The cytoplasm is cleaner and the chromosomes sharper than in preparations de-stained with iron-alum.

Good results have been obtained with brazilin which was introduced by Belling (5) and Capinpin (10). This stain, apparently little used, is superior in some respects to haematoxylin (which it most closely resembles), especially for early stages of meiosis in smear preparations. The method has the advantage that tissues are never in water and maceration is, therefore, avoided; the chromosomes are sharply defined, even when the cytoplasm is slightly stained. The stain appears to be permanent and seems to work best after Navashin fixations. To de-stain thick sections is difficult, as in the case of haematoxylin.

The Feulgen reaction (24) was originally developed as a micro-chemical test for distinguishing a type of nucleic acid present in chromatin. In consequence, it has been used by various workers for detecting chromatin, and more recently as a general stain (see schedule) (28, 30, 64). Success depends on the type of basic fuchsin used, for many types do not de-colorise sufficiently and are, therefore, useless. The length of time necessary for hydrolysing in N/HCl is also important. Bauer (4) finds that hydrolysis for a certain length of time gives different results after different fixatives. He gives a useful graph showing the optimum times required after Navashin, Carnoy and some other fixatives. The most intense staining is obtained after fixation with Navashin's fluid, although this is not as satisfactory for chromosome morphology as a fixative containing osmic acid.

It often happens that when slides are placed in water or in alcohols of low concentration some of the stain remains in the cytoplasm, so that the final result is blurred and indistinct. Heitz (30)

in his method omits the sulphide washes; he finds that by using 45% acetic acid or 96% alcohol, directly after staining, the stain never remains in the cytoplasm. This has been confirmed by Klingstedt (unpublished) who finds 45% acetic acid eminently satisfactory on insect material. Klingstedt uses a technique somewhat similar to that used by Heitz for root tips (30). The application of this method to pollen mother-cells is being investigated.

A full discussion of the problems involved in the Feulgen technique and of methods for improving it has recently been published by de Tomasi (21), and Carlson (11) mentions it in reference to the effect of fixatives on staining reactions. It seems likely that the Feulgen reaction will be widely used if some of the present difficulties can be overcome.

Schedule 5.—*Gentian-violet-iodine method*

STAIN 3-10 minutes in 1% aqueous solution of gentian-violet or crystal-violet, boiled and filtered.

RINSE in water.

PASS THROUGH IODINE: 1% iodine + 1% KI in 80% alcohol, 30-45 secs.

Rinse in 95% alcohol.

Wash in absolute alcohol, 4-10 secs.

DIFFERENTIATE in clove oil under the microscope, about 30 secs.

WASH in xylol, 3 or 4 changes, for at least 10 mins.

MOUNT in balsam.

Note: The best results are obtained by adjusting the length of time in the stain so that differentiation is as rapid as possible. A .1% aqueous solution can be used with advantage for large chromosomes in pollen mother-cells.

The slides should be thoroughly washed in xylol after differentiation, as the slide will fade rapidly if any clove oil remains.

Schedule 6. *Tentative Feulgen Staining Procedure*

1. *Preparation of staining solution.* Dissolve .5 gm. of basic fuchsin by pouring over it 100 cc. of boiling distilled water. Shake thoroughly and allow solution to cool to 50° C. Filter and add 10 c.c. N/HCl to filtrate. Cool to 25° C. and add .5 gm. anhydrous sodium bisulphite. The solution must be kept in the dark and allowed to stand 18-24 hours before using; it should then be either colorless or light straw color.

2. Run the slides down to water and hydrolyse in N/HCl at 60° C. for:

8-25 mins. after Navashin fluids

14-25 " " Flemming solutions

12-25 " " 2 BE, 2 BD.

3. Rinse slides in distilled water and stain in the decolorised fuchsin. Plant tissues 3-5 hours, animal 2 hours.

4. Transfer slides direct from stain into 45% acetic acid. 3-5 minutes are sufficient.

5. Pass slides through the following solutions:

1 part absolute alcohol : 1 part acetic acid

3 parts " " : 1 " " "

9 " " : 1 " " "

Pure absolute alcohol

1 part absolute alcohol : 1 part xylol.

2 to 3 minutes in each solution are sufficient.

5. PRE-TREATMENT

Special techniques have been devised by various workers for the demonstration of spiral structure in chromosomes. These methods depend on coagulating the chromosomes in such a way that a staining thread is separated from a non-staining matrix (19). The most successful methods are those involving pre-treatment with ammonia fumes (37, 39) or ammonia in alcohol (54) or acid fumes (39). Fixing with hot water has also given successful results (52, 39). Nebel (48) has demonstrated the effects of special fixatives and of desiccation. Huskins and Smith (31) also found that desiccation revealed the spiral structure in *Trillium*. Brieger (unpublished) finds that immersion of anthers for 15 to 45 minutes in 5% solutions of nitrates of most common salts except calcium before fixation will show coiling in *Lilium* and *Paeonia*. Goodspeed and Uber (25) made use of the Altmann freezing and drying technique for demonstrating internal structure in chromosomes.

The procedure of Kuwada and Nakamura is as follows. Cells of an anther at the metaphase or anaphase stage are teased out on a slide in a small drop of 3% cane-sugar solution. The slide is then inverted for a few seconds over a cylinder containing ammonium hydroxide (880 vols.). The excess sugar solution is removed with blotting paper before fixing and staining in aceto-carmine. The staining after this treatment is never very intense.

The method has since been modified by La Cour (39) to enable permanent smear preparations to be made with Flemming or Navashin fixation, thus allowing the use of a more selective stain like gentian-violet. Anthers of the stage required are smeared on a slide which is immediately immersed, smeared side down, for a second or two in a dish of 3% cane-sugar solution. The slide is then laid flat for 5 to 10 seconds over a jar containing ammonium

hydroxide 880 vols., as before. The preparation may then be fixed and stained like an ordinary smear. Ammonia vapour may be replaced by the fumes of acids, with similar results. Of the acids tried, nitric gave the best results. Careful handling is required to prevent loss of cells when the slide is immersed in the sugar solution, for this does not coagulate the cells and make them stick to the slide as a fixative does. The loss is eliminated to some extent in the permanent method of Sax and Humphrey by the use of weak alcohol, which coagulates the cells. A smear is made in the ordinary way, but before fixing, the slide is placed for a few seconds in a 30% solution of alcohol to which a few drops of ammonia have been added. The time of fixation in this method is shortened to 3 to 10 minutes. The slide is then rinsed in 40% alcohol and placed in 60% for 30 minutes or longer before staining.

The results obtained by the two methods are similar. Coiling can be seen in 50% to 80% of the cells. Gentian-violet, the Feulgen stain or haematoxylin may be used on slides treated by these methods, with similar results. Huskins and Smith (31), however, report that *Trillium* chromosomes subjected to slight desiccation before fixation and stained by the Feulgen stain do not show spirals.

6. ILLUMINATION AND PROJECTION

It is impossible to deal adequately with the science of optics in a review of this kind. The reader is, therefore, referred to the work of Belling (7) who has made an important contribution to our knowledge of this subject. I propose, however, to deal briefly with the question of illumination.

A microscope lamp designed for Belling's method of illumination has been described by Waterman (62), using a two-filament bulb and a reflector. A condenser is unnecessary. Many workers find the small motor headlight bulb a cheap and efficient illuminant. A simple lamp may be made using a 12 volt 40 or 60 watt headlight bulb.

Illumination by gas is cheap, but as a rule it is variable and not very intense. Probably the thorium oxide pastille affords the best method. In order to obtain an even field, a flat circular area of thorium oxide must be presented to the flame. The thorium, however, becomes increasingly brittle with use, and fragments drop off, so that a flat surface cannot usually be maintained for more than

a few months. In spite of its disadvantages this method is very satisfactory.

In all cases where an intense light is used, a green screen improves the definition and reduces the eye strain. The screen should be neither too green nor too yellow. It should make chromosomes stained in gentian-violet look black without too greatly reducing the intensity of the light.

A projection method for demonstrating large chromosomes *in situ* to a limited audience (10-15 persons) has been published by Darlington and Osterstock (20). A 10 ampere arc lamp with suitable condenser gives sufficient light for projecting at a distance of 9 feet, using a $\times 90$ apochromatic oil immersion objective N.A. 1.3 and a $\times 5$ eye-piece. A water-ammonia heat-absorbing trough must be placed between the light and the inclined microscope to prevent injury to the slides and to the lenses. Experiments with various screens, silver, bead, ground glass and plain white, showed the last to be the most satisfactory.

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RECENT WORK ON PHOTOPERIODISM

W. W. GARNER

Bureau of Plant Industry, U. S. Department of Agriculture

In the 16 years which have elapsed since the appearance of the first paper of Garner and Allard on photoperiodism in 1920, there has been a rapid accumulation of literature dealing with various phases of the subject. Much the greater portion of this research, especially in the earlier years, has been directed toward broad development of the numerous forms of visible response to relative length of day and night, as observed in a very large number of species, but with considerable emphasis on initiation of sexual reproduction, the formation of tubers and related structures, interrelationship of the light period and other factors of the environment, and the comparative responses of closely related forms differing as to the geographical latitude of their origin. The literature on photoperiodism through the year 1932 has been reviewed elsewhere by the writer (15) and earlier reviews have been published by Schick (34), Redington (30) and Kellerman (17), so that the present discussion will be limited chiefly to developments in the subject during the past 5 or 6 years. It would lead too far afield to attempt to cover all phases of photoperiodic response or to review within the space available all papers which have appeared, even with respect to those features dealt with. As a whole, the new work during the past half dozen years clearly shows a trend toward study of the mode of action of the light period, this problem having been approached from various angles, and in some instances, with very interesting results.

PHOTOPERIODIC CLASSES

It seems desirable at the outset to refer briefly to the now rather well-known classification of plants into three groups on the basis of their photoperiodic response with reference to flowering and fruiting, namely, the short-day, the long-day and the day-neutral types. Perhaps the particular contrast in response to day length on which this classification rests, constitutes the most important single feature of photoperiodism and it clearly furnishes a major point of attack in attempting to determine the mode of action of the daily

period of light (photoperiod). In the short-day type of plant, reproductive activity is initiated or accelerated by a relatively short daily period of light, while the long-day type responds similarly to a relatively long photoperiod. The situation essentially is that the short-day plants readily flower and fruit through a wide range in daily hours of illumination up to, but not above, a certain maximum known as the critical daily light period, while the long-day plants flower and fruit through a wide range in daily hours of light (including even continuous illumination) down to but not below the critical light period (14). Short-day plants, accordingly, tend to remain vegetative with day lengths in excess of the critical, and long-day plants continue vegetative activity with day lengths less than the critical. The critical photoperiods for short-day plants, as a class, do not differ greatly from those of long-day plants and, in fact, the two frequently overlap. The critical periods for a large proportion, though by no means all, of the plants of both types fall within day lengths of 12 to 14 hours.

Obviously, the classification of plants into long-day and short-day types does not depend merely on differences in the critical photoperiod itself, but rather on whether it marks the lower or the upper limit of day length conducive to reproductive activity. Those plants which flower readily under either a long or a short photoperiod and are designated as the indeterminate or day-neutral type, of course, have no critical photoperiod. These principles of classification are considered in some detail in a recent contribution by Kopetz (19) dealing largely with the relation between the critical day length and the seasonal long-day, short-day and day-neutral reaction of summer annuals. It is pointed out that the long-day type will have a vegetative stage of minimum, constant duration if growth from the outset falls within the period from spring to fall, while the short-day type will have a pre-flowering stage of minimum, constant duration if growth falls wholly within the period from fall to spring. These conclusions, of course, are based on the assumption that for the seasons indicated the day length will not fall below the critical for the long-day type, nor above it for the short-day type. It is considered by Kopetz that the critical day length for any species or variety is subject to modification by change in the intensity and the spectral composition of the radiation.

In connection with a recent study of photoperiodism in relation to frost resistance in perennials, Moshkov (24) concludes that since photoperiodic conditions influence the whole life activity and all principal characters of the plant, including such an important character as frost resistance, a plant should not be classed as day-neutral merely because day length does not influence the transition from the vegetative to the reproductive stage. For this reason the present system of classification is regarded as inadequate. Once the internal mechanism of the length of day effect is sufficiently understood, it may be possible to devise a more comprehensive classification which would meet the objection raised by Moshkov, but in the meantime the present system, despite its limitations, seems to serve a useful purpose as a convenient means of contrasting the photoperiodic response of the plant groups in question with respect to transition from the vegetative to the reproductive stage.

VARIETAL DIFFERENCES IN PHOTOPERIODS

The recent work of Evans and Allard (10) with timothy well illustrates the marked differences frequently existing in the critical photoperiods of otherwise closely related varieties and strains of economic species, a situation which has often been overlooked by investigators who have reported results obtained with many species without identifying the variety or strain worked with, making satisfactory interpretation very difficult. In the present case, a collection of 16 strains of timothy of American and North European origin, which under natural conditions in the vicinity of Washington, D. C., range from very early to very late in flowering and ripening of seed, were grown with photoperiods ranging from 10 to 18 hours. Under the natural length of day, with a maximum length of $14\frac{3}{4}$ hours, the date of first flowering in the various strains ranged from June 3 to July 27, whereas under an 18-hour day all flowered June 3-8. The minimum photoperiod required for earliest flowering in the different strains ranged from 12 to 17 hours, and the critical light periods appeared to range from 10 hours or less to 15 hours. It is concluded that earliness or lateness of different strains of timothy is chiefly a matter of adaptation to day length, the earlier ones being adapted to a relatively short day.

TUBERIZATION

That length of day is an important factor in tuberization was shown in earlier publications of Garner and Allard and others. It

will be of interest to consider here the work of Rasumov (27) and of Hackbart (16) on the influence of the photoperiod on growth and development in tuber-forming species in relation to the latitude of their origin. It was found by Rasumov that several species normally yielding good crops of tubers in their native habitat in the equatorial region of South America formed no tubers at all when grown in Leningrad during the long days of summer. In tests with regulated photoperiods, *Solanum tuberosum* was indifferent to this factor, so far as concerns initiation of tuber formation, while a long photoperiod caused considerable delay in tuber formation in *S. andigenum* and brought about complete suppression of tuberization in several other species of *Solanum* and certain tuber-producing species of *Ullucus*, *Oxalis* and *Tropaeolum*. All species, without exception, showed maximum vegetative development and usually flowered abundantly under long-day conditions, while the short day was optimum for tuber formation. Working also with material derived from South America and basing his classification on weight of tubers produced per plant, Hackbart found that in Germany forms of *Solanum andigenum* from the region between 4.5° N. and 12° S. were almost exclusively of the short-day type, but with increase in latitude southward the day-neutral forms gradually increased and at 20°–33.75° S. became dominant. A form of *S. tuberosum* from the Island of Chiloé, 42.5° S., responded as a long-day type. Aside from the matter of photoperiodic response in relation to geographical latitude of origin, there is more or less general agreement in these results of Rasumov and Hackbart with those of various other workers pertaining to general features of tuberization which go to show that usually a long photoperiod favors extensive top development and limited production of tubers, while a short photoperiod is favorable for maximum tuberization when comparison is made with the accompanying restriction of top growth, and an intermediate photoperiod gives the maximum absolute production of tubers. Accordingly, it is apparent that with a decreasing photoperiod the growth rate is curtailed more rapidly than is the formation of carbohydrate. However, in the case of the onion, bulbing is favored by a long photoperiod and vegetative activity is promoted by a short photoperiod.

PHOTOPERIODICITY IN WOODY PLANTS

Probably because of the experimental difficulties involved, comparatively little work has been done on flowering response of woody

plants to length of day. However, Allard (1) has shown that such plants are capable of responding to photoperiod in the same manner as herbaceous species. Shrub-althea, *Hibiscus syriacus* L., was found to be a long-day type with a critical photoperiod of 12 hours or slightly less. Flowering was suppressed for the four years of the test by a 10-hour day but was abundant with photoperiods of 13 hours or longer. Bougainvillea, *Bougainvillea glabra* Choisy, responded as a short-day type, flowering abundantly with a 10-hour photoperiod. Its critical photoperiod appears to be somewhat in excess of 12 hours. Turkscap hibiscus, *Malvaviscus conzattii* Greenman, proved to be day-neutral in the latitude of Washington, D. C.

Results with *Sesamum* reported by Rhind (31) in Burma, with an annual range in day length only from somewhat less than 11 to $13\frac{1}{2}$ hours, emphasize the fact that photoperiodism is an important factor in plant growth even at low latitudes. Distinct "early" and "late" forms of *Sesamum* are recognized in Burma, the latter making, when planted early, a rank growth with little flowering and no fruiting, but producing normally if planted late. The late forms proved to be typical short-day plants, growing and reproducing abundantly when exposed to a photoperiod of 12 hours or less. These results are in line with the general rule that late summer and fall flowering strains and varieties are short-day types, while the early sorts of the same species are likely to simulate the day-neutral type.

Considerable evidence has been supplied by recent investigations in support of earlier work indicating that photoperiodic response is an important factor in the periodicity of growth of woody plants and in their adaptation to different latitudes. At Leningrad, Bogdanoff (2) subjected 1-2-year-old seedlings of broadleaf and coniferous species to the normal day and regulated photoperiods of 13 and 9 hours under outdoor conditions. With a shortened day length the period of growth of all species was reduced and subsequently there was earlier emergence from dormancy. Larches from southern latitudes showed very late growth under the normal day, but under a short photoperiod vegetative activity ceased 6 weeks earlier. Southern forms of pine made scant growth under the normal northern day and were injured by winter cold, whereas exposure to a shortened photoperiod produced good growth and

the trees stood the winter well. Employing similar methods, with addition of supplemental artificial illumination and localized treatments of the plants, Moshkov (24) found that frost resistance depends on photoperiodic response, a favorable photoperiod applied for only 20 days being adequate for some species. It sufficed to expose only the top of a plant to the proper photoperiod to induce frost resistance in the primary stem. There is a critical photoperiod for frost resistance and beyond the limits of this critical range differences in the light period may be wholly without effect. Differences in frost resistance of a given species in different latitudes are determined primarily by photoperiodic conditions during vegetation.

Kramer (20) observed that when exposed to the normal length of day, all of 10 broad leaf and coniferous species tested ceased growth in autumn as early in a warm greenhouse as out-of-doors. These species were white ash, *Fraxinus americana* L., green ash, *F. pennsylvanica* var. *lanceolata* Sarg., beech, *Fagus grandifolia* Ehrb., black locust, *Robinia pseudoacacia* L., yellow poplar, *Liriodendron tulipifera* L., red gum, *Liquidambar styraciflua* L., post oak, *Quercus stellata* Wang, northern red oak, *Q. borealis maxima* Ashe, white oak, *Q. alba* L., and loblolly pine, *Pinus taeda* L. With a fixed photoperiod of 14½ hours, all species except white ash, green ash and red oak grew longer and more rapidly than with the normal length of day. Loblolly pine grew all winter with a photoperiod of 14½ hours, as did yellow poplar with a 16-hour photoperiod, while these species together with red gum behaved similarly with continuous light. Resumption of growth after dormancy was hastened in beech, yellow poplar, red gum and red oak by a lengthened photoperiod and was retarded by shortening the photoperiod below the normal.

PHOTOPERIODIC AFTER-EFFECT

The photoperiodic after-effect which comes into evidence when the plant is first exposed to a short day for a limited period and subsequently transferred to a long day, or *vice versa*, has received considerable attention in recent years, largely because of the fact that except at the equator the photoperiod experienced by the plant in nature is constantly changing. It was shown by Garner and Allard (13) that when typical short-day plants were first exposed to a short photoperiod for 10–12 successive days and then trans-

ferred to a long photoperiod, sparse flowering subsequently occurred, although there was considerable delay due to the superimposed retarding action of the long day. A pre-treatment of less than 10 days was without effect on time of flowering and a minimum pre-treatment of 21 days with the short photoperiod was necessary for successful fruiting. Extending his earlier work in this direction on flowering to the process of tuberization, Rasumov (27) found that pre-treatment with a long photoperiod so as to afford extensive vegetative growth, followed by exposure to a short photoperiod, commonly results in maximum yields of tubers. When the order of treatment is reversed, the carry-over effect of the short photoperiod induces tuberization in cases in which tubers are not normally produced under the long photoperiod, although the yield is poor. Moreover, in several species the influence of the subsequent long day is so strong that the tubers formed from the carry-over effects of the short day tend to revert to stolons and give rise to new shoots. Rudorf (32) exposed numerous sorts of soybean and bush-bean alternately to various combinations of the natural day of 16-17 hours and photoperiods of 8, 10 and 12 hours. It was found that pre-treatment with a photoperiod of 8 hours for as brief a time as 10 days may determine the future development of the plant, although it does not correspondingly restrict vegetative growth. The after-effect of the short photoperiod can not be due to increased carbon assimilation since the reserves of the seed are at a maximum at this stage and it is suggested that enzymes or hormones formed by the short photoperiod are involved. It was found by Lubimenko and Sceglova (22) that the carry-over effect of a brief pre-treatment with a 6-hour photoperiod in hastening flowering in soybeans is not materially affected by intensity of the illumination. In fact, the same effect was obtained by an initial exposure to continuous darkness for 10 days. Čajlachjan and Alexandrovskaja (7) have pointed out definite limitations which apply to the after-effect as a feature of photoperiodism. Experimental evidence is presented which suggests that a true photoperiodic after-effect is seen only in the acceleration of reproductive processes and development of the organism. For this reason only short-day after-effect is possible for short-day plants and long-day after-effect for long-day plants. A long day produces no carry-over effect on short-day plants and a short day produces none on the long-day type. There is consider-

able evidence in the earlier literature which appears to support this view.

VERNALIZATION

Of the several environmental factors commonly influencing plant growth and development in association more or less with the effects of the photoperiod, special prominence has recently been given to temperature because of its rôle in the phenomenon known as vernalization. Quite apart from the latter, moreover, there is a rather close parallelism in the seasonal change in day length and in temperature so that in many cases the two factors are likely to be simultaneously operative. Vernalization, "the conversion of a winter plant into a spring plant," can be referred to here only very briefly in its relation to day-length requirements. It appears that by such pre-treatment the light requirements of the plant may be modified, at least in some instances, but this does not mean that definite photoperiodic requirements for subsequent growth and development are thereby dispensed with. Recent work of Lebedinseva (21), Purvis (26), McKinney and Sando (23) and others, having to do with the relationship between conditions of germination and subsequent light and temperature requirements, indicate the following situation with respect to winter and spring cereals. Short-day conditions tend to inhibit completion of the life cycle in both types regardless of pre-treatment during germination. In winter cereals the early stage of flower differentiation is delayed by a long day but subsequent development is favored, whereas the spring cereals respond favorably at all stages to the long day. Vernalization, however, causes the winter type to follow the spring form in response to day length. Spring cereals have relatively long day and high temperature optima throughout their life cycle while the winter forms have short day and low temperature optima during the early phases of growth but optima similar to those of the spring varieties in later phases of growth. According to Čajlachjan (3), vernalization of winter plants can be accomplished with light as the active factor if the temperature is relatively high and the supply of moisture is adequate. Quick flowering was obtained in midsummer plantings of winter wheat, rye and other plants when grown with continuous light. At present, the evidence pertaining to the effect of seed treatment on the subsequent day-length re-

quirements of short-day plants is conflicting, but it appears that in some cases the effect is to promote flowering under a long day or continuous light.

In sand cultures of a strain of potato of medium earliness conducted in the greenhouse, Werner (36) found that a high temperature, long day and abundant nitrogen supply favored vegetative growth in all parts except the tubers. Maximum tuberization was obtained with a low temperature, abundant nitrogen and an intermediate day length while a short day gave the highest ratio of tubers to tops. With conditions favoring tuberization, lack of nitrogen did not accelerate tuber formation but inhibited vegetative growth. Darrow (9) finds that the ordinary varieties of strawberries are short-day types, usually forming flower buds only under the short days of fall in combination with cool temperature while a long day induces runner formation and an intermediate day length tends to produce branch crowns. The ever-bearing sorts are classed as long-day types, forming their flower buds under the long days of high latitudes. The regional adaptation of varieties is dependent upon their specific day-length and temperature requirements. As a result of extensive observations on *Beta vulgaris* L., Chroboczek (8) found that a temperature of 50–60° F. and a photoperiod of 15 hours or more are most effective for seed production, and favorable conditions of temperature and light are essential to development of a fertile inflorescence.

Steinberg and Garner (35) have recently reported observations on early-, medium- and late-maturing varieties of soybeans grown at four different temperature levels and with various photoperiods under accurately controlled conditions. Despite the use of the tungsten filament lamp as the light source, the reproductive responses of the three varieties to the photoperiod and to temperature agreed closely with previously reported results with natural illumination, a matter of some importance from the standpoint of possible effects of the quality of the light on the response to day length. There were only slight differences in temperature requirements but at each temperature there was definite contrast in the three varieties with respect to the critical photoperiod for flowering. Seasonal change in day length is the controlling factor in the contrast in time of reaching maturity shown by the different varieties. The total light hours from germination to flowering increased with increase

in length of the photoperiod. While soybean is distinctly a warmth-loving species of the short-day type, sugar beet in similar tests proved to be a long-day type in which flowering is favored by a lower temperature level. However, in each case, within the temperature range favorable to reproductive activity, flowering was hastened by increase in temperature.

ARTIFICIAL ILLUMINATION

Some remarkable results have been reported on the minimum intensity of supplemental artificial illumination required to produce definite photoperiodic response by prolonging the natural daily period of light. It has been shown by many investigators that for this purpose intensities of the order of 5-10 foot candles are ample for many plants and it has been rather generally agreed that photosynthesis could not be directly concerned in the response. Moreover, in recent experiments Withrow and Benedict (37) obtained early flowering in *Callistephus chinensis* with supplemental light of an intensity of .1 foot candle, which is stated to be only twice the intensity of bright moonlight. The supplemental illumination was so applied as to extend the winter daily light period to 21 hours. Equally surprising is the fact that the supplemental illumination at an intensity of .3 foot candle produced a distinct increase in dry weight of the plant, not only in aster but also in *Viola tricolor* and *Matthiola incana*. The supplemental illumination amounted to not more than 3 foot candle hours per night as against a minimum of 5000 foot candle hours supplied by the basal solar radiation per day. Going a step further in the formative action of very weak supplemental light, Gaertner and Braunroth (12) obtained earlier flowering by a few days in the 4 long-day species, *Hordeum distichum*, *Triticum vulgare*, *Iberis amara* and *Agrostemma Githago*, by utilizing bright moonlight as supplemental illumination and a similar delay in flowering in the short-day types, *Soja hispida* (*Soja Max* (L.) *Piper*) and *Pharbitis hispida*. The intensity of full moonlight is usually considered as being .02 foot candle. These results, of course, emphasize the importance of the factor of daily duration of light in plant growth and development and obviously have a direct bearing on the problem of the mechanism involved.

SPECTRUM INVESTIGATIONS

As an approach to the mode of action of the photoperiod several investigators have recently turned their attention to the photoperi-

odic effects of different regions of the visible spectrum. In most instances the plants were exposed to white light for a portion of the day followed by exposure to colored light instead of darkness for the remainder of the day. Apparently in all cases the intensity of the colored light used has been low. With both long-day and short-day types of plant, Rasumov (29) found that with 10 hours daily of sunlight or white artificial light and 14 hours of colored light, supplemental illumination of the longer wave lengths, especially the red, acted as white light while green, blue and violet light acted like darkness, so far as concerns reproductive processes. By increasing the proportion of red rays at the expense of the shorter wave lengths the vegetative period was progressively shortened in long-day plants and prolonged in the short-day types. However, varieties and species were found to vary as to their sensitivity to differences in wave length of the light.

Employing colored lights of equal energy values, Schappelle (33) finds that when used to prolong a natural short day, red and blue are about equally effective in inducing reproductive response in the long-day types, radish, spinach, *Crepis* and *Marchantia*. Blue light is superior to red for lettuce, probably because of its better effect on the vigour of the plant. In the short-day plants, *Salvia*, *Chrysanthemum*, *Kalanchoe*, Maryland Mammoth tobacco and teosinte, the blue, red and white lights all were effective in suppressing flowering when used to extend a natural day of 10 hours. However, 5 hours of red light following 5 hours of daylight, or 10 hours of daylight alone, produced good flowering response, whereas 5 hours of blue light following 5 hours of daylight largely suppressed reproductive response.

In tests with wavebands of red, green and blue of relatively balanced energies to prolong the normal winter day to 18 hours, Withrow and Biebel (38) obtained early flowering in the long-day types, *Callistephus chinensis* and *Helianthus cucumerifolius*, but not in *Scabiosa atropurpurea* with the blue radiation; all flowered early with the red; with the green radiation, flowering was delayed in *Callistephus* and *Helianthus* and did not take place at all in *Scabiosa*. The short-day plants, *Salvia splendens*, *Cosmos bipinnatus* and *Tithonia speciosa*, remained vegetative with the supplemental red light but flowered when the blue and green were used to lengthen the day. *Salvia* already in the flowering condition reverted to the

vegetative stage under the red but not under the blue and green lights. Funke (11) treated the short-day types, *Solidago virgaurea*, *Cosmos bipinnatus*, 3 species of *Aster* and 8 varieties of *Chrysanthemum indicum*, with 8 hours of day light supplemented with 16 hours of red, blue and white lights. In *Solidago*, *Cosmos* and *Aster* the blue light acted much the same as darkness but the red produced about the same results as the white light in delaying flowering. In *Chrysanthemum* the results with the blue were intermediate between those of darkness and the red or white light. These results as a whole clearly indicate that at relatively low intensities the longer wave lengths of visible radiation are more efficient than the shorter wave lengths in bringing about normal photoperiodic response in both the long-day and the short-day types of plants. It has not been shown, however, that any particular portion of the spectrum is essential.

VEGETATIVE VS. REPRODUCTIVE DEVELOPMENT

Some interesting and seemingly important evidence bearing directly on the mechanism or mode of action of the photoperiod on plant development has been just recently brought to light, mainly with respect to the location in the plant and the nature of the initiating processes concerned in the transition from the vegetative to the reproductive state. The fact that by appropriate treatment photoperiodic response may be strictly localized in individual parts of the plant was demonstrated by Garner and Allard and subsequently has been confirmed by others. Although evidence previously had been presented by Knott indicating that the response may be sharply localized in the apical growing point, his more recent results (18) suggest that although visible response may be in the bud the foliage leaves function in some manner in the response. It was found that seed-stalk formation was not induced in spinach, a long-day plant, when the apical bud was exposed to a long day and the leaves to a short day, but did take place when the light treatments were reversed. Apparently the hastening effect of the long day on flowering was exerted through the leaves.

Čajlachjan (4, 5, 6) has reached a similar conclusion as a result of localization experiments with both long-day and short-day plants in which the principle of the photoperiodic after-effect was utilized. In the experiments of Garner and Allard as well as in the confirma-

tory work of Čajlachjan the photoperiodic reaction was localized in a branch or a shoot having on the main stem both leaves and apical or axillary growing points. In further tests by the latter, millet, a short-day type, and barley, a long-day type, were grown for an initial period of 20 days under both long and short days. In different lots of each series of plants a varying number of leaves ranging from none to all were removed from the plants, as they appeared. At the end of the 20-day period the millet plants under the short day were transferred to the long day alongside of similar plants exposed to the long day right along and barley plants were similarly shifted from the long to the short day. Response of the plants to change in day length diminished with decrease in the leaf surface left intact, there being no response where no leaf surface remained. Chrysanthemum plants were decapitated and only 3 upper shoots were allowed to remain, all leaves being removed from the latter while 8 leaves were left on the primary stem below. In one series the leafless shoots were exposed to a 10-hour day and the lower portion of the primary stem and its leaves were exposed to the normal day while in a second series these treatments were reversed. After a month all plants received the normal day. In the second series flower buds began to appear in 10 days but in the first series the buds were nearly 3 weeks later in appearing. These responses were in agreement with those of controls in which the whole plants were exposed to the short and long day, respectively. In a second test with chrysanthemum only one leaf together with its axillary shoot was left on the stem. In one instance both the leaf and the stem were exposed to the long day and in the other the leaf was given a 10-hour day as the remainder of the plant received the long day. In the first case the shoot remained vegetative but in the second it produced flower buds. From the results, Čajlachjan concludes that the processes induced by changes in the length of daylight and leading to flowering and fruiting occur within the leaf tissues, and it is considered that transfer of the influence of these processes to the growing point may be accomplished only by material carriers capable of being transported and of controlling the development of the shoot.

Again, experiments with various short-day plants have shown that the growth of stem and leaves and the accumulation of dry matter increase with increase in day length but flowering is hastened

by an artificially shortened day. As to long-day plants, although initially both types of activity are promoted by a long day, ultimately after flowering has occurred under the long day more vigorous growth results under a short day, yet no flowers are formed. Consequently, processes of development occur independently of processes of growth and the material transmitters of the light effect on the leaves are not nutritive materials but specific substances having a regulatory function, that is, hormones. The usual movement of flower hormones is from the leaves to their axillary shoots though a certain portion moves both upward and downward through the stem to the growing zones. When each alternate leaf of *Chrysanthemum* is exposed to a short day the axillary shoots of these leaves readily produce flowers but no open flowers appear on the shoots of the remaining leaves exposed to a long day, though rudimentary buds develop.

Pushing his experiments still further, Čajlachjan found that when partially defoliated tops of *Perilla nankinensis* plants, grown under long day conditions, were grafted on stumps of other similar perilla plants, flowering occurred in the axillary shoots of the stocks exposed to a short day while there was no flowering in the scions exposed at the same time to a long day. However, if the flowering shoots of the stocks were constantly removed flowering soon occurred in the scions. Therefore, there was a movement of blossom hormone from the leaves of the stock into the growing points of the grafted tops. As long as the axillary shoots remained these received most of the hormone from the leaves. Finally, when tops of the short-day plant, *Helianthus tuberosus*, grown under long-day conditions and therefore strongly vegetative, were grafted on stumps of *Helianthus annuus*, a neutral-day plant already in the flowering condition, flower buds appeared on the *tuberosus* scions in due season if the lateral flower shoots of the *annuus* stocks were stripped off. Hence, the flower hormone is not specific in its action for separate plant species, its nature being the same in different plants. For the flower hormone, the physical and chemical characters of which as yet are unknown, Čajlachjan proposes the name *florigen*.

It has been recently suggested, also, that hormones are concerned in the influence of day length on tuberization. In a study of localization phenomena which occur when different parts of tuber-form-

ing plants are subjected respectively to a long and a short photoperiod, Rasumov (28) found that there is no specific organ for reception of the tuber-forming stimulus which is supplied by a favorable day length. However, the apical growing point was recognized as playing an important role in reception of the stimulus. Zimmerman and Hitchcock (39) obtained tuber formation and checking of stem elongation in *Helianthus tuberosus* when the stem tips were exposed to a short day, just as when the whole plant was given a short-day exposure, whereas control plants exposed to full day produced only rhizomes and no tubers. The results indicate that the growing stem tips exercise a regulatory influence on development of underground stems and tubers. The regulators probably are hormone-like substances produced in the stem tips and are transported to other parts of the plant where they exert a controlling influence on development.

Murneek and Gomez (25) have recently reported results of histological observations on the comparative development of the main stem and axillary buds in the Biloxi variety of soybean, a short-day type, when grown under day lengths of 7 hours and 14 hours. At the end of 21 days the promeristem of the 7-hour day plants was distinctly conical in form, whereas under the 14-hour day it was somewhat cylindrical, forming a plateau at the apex*. There was a gradual, progressive decrease in size of the promeristem in the former plants, while in the latter, decrease in size of promeristem did not take place till much later. In plants of long-day exposure there was marked delay in rate of formation and maturation of meristematic cells, thus permitting initiation and further growth of the primordia of leaf and other organs, although differentiation of the histogen occurred soon after the appearance of leaf initials. The promeristematic and meristematic cells were larger but fewer in number under the short-day exposure. Up to the 21st day, development of the axillary buds was similar under the two exposures. The earliest indication of initiation of flower buds was in 14-day old plants under the short day. These buds appeared in the axils of the older leaf initials on the lowermost axillary shoots. Unmistakable signs of flower buds were evident by the 21st day, including appearance of the bract. Open blossoms appeared on the 42nd day. Under the long day, the plants remained vegetative.

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THE BOTANICAL REVIEW

VOL. III

JUNE, 1937

No. 6

RECENT FLUCTUATIONS IN PLANT DISEASES IN THE UNITED STATES

NEIL E. STEVENS

University of Illinois

and JESSIE I. WOOD

Bureau of Plant Industry

INTRODUCTION

Whatever may be the economic effects of variations in the losses from plant diseases, their fluctuations, both in extent of area covered and in severity within given areas, add greatly to the interest of their study. Much of our information regarding the incidence of plant diseases in the United States is contained in the publications of the Plant Disease Survey, from which most of the material used in this paper has been drawn. It is the purpose of the present review to bring together the available information regarding the extent of the fluctuations of certain diseases in the United States prior to 1936. Little consideration will be given to specific environmental factors which may have caused these variations, although some reference is made to the possible results of control measures, and the apparent economic effects of variations in losses are briefly considered.

Choice of the diseases included and the length of period covered has been determined by what is known concerning the reliability of the information available. Two of the diseases considered—downy mildew of tobacco and bacterial wilt of maize—have been the subject of much observation by plant pathologists in various states, and their information has been supplemented by intensive surveys by members of the Bureau of Plant Industry. The recently introduced Dutch Elm disease and the wasting disease of eel grass (*Zostera marina*), which, if not new, has no adequately recorded history, are necessarily excluded from this discussion.

DOWNY MILDEW OF TOBACCO

Downy mildew, caused by *Peronospora tabacina* Adam, has long been known as a serious disease of tobacco (*Nicotiana tabacum* L.) in Australia. It was first discovered in the United States in 1921 (9) in seed beds of the shade tobacco area around Quincy, Florida (Fig. 1), and in the nearby tobacco-growing region of Georgia. American students of plant disease and the American public as well, had by this time, through the object lesson of the chestnut

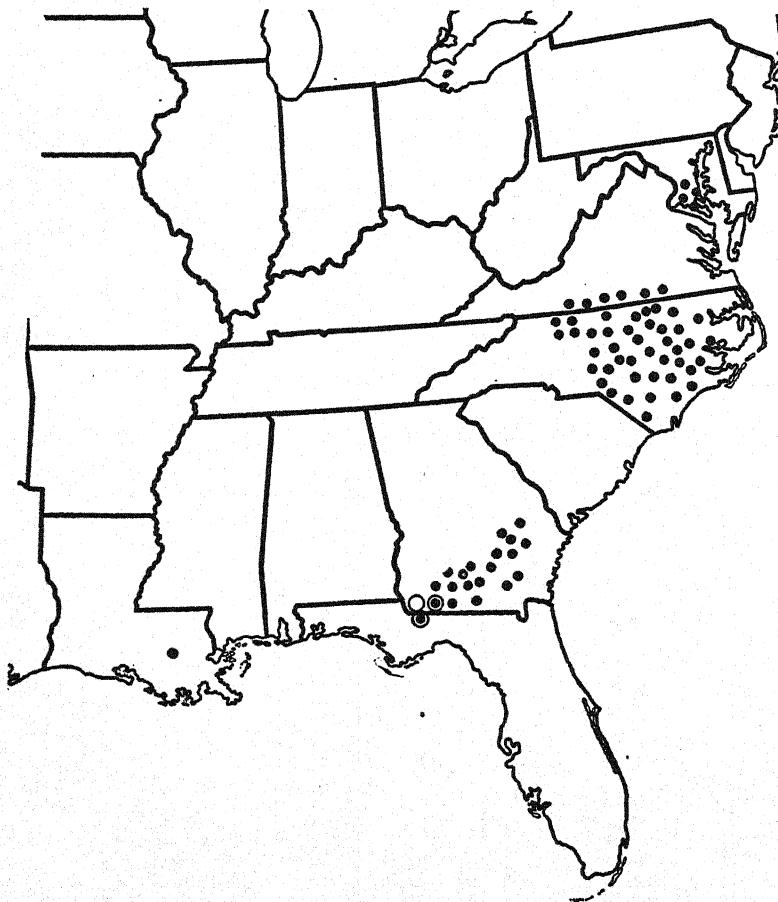


FIG. 1. Distribution of downy mildew of tobacco in 1921 and in 1931. Circles indicate location of counties in which disease was found for the first time in 1921. Dots show location of counties in which disease was found in 1931.

blight, become thoroughly aroused to the potential danger of an introduced plant disease. Predictions were freely made of the dire results which would follow this latest introduction—predictions which resulted in a flurry on the stock market. Actually, however, the disease did little damage in 1921 and was not seen again in the United States for ten years.

The 1931 outbreak was much more serious. The disease first appeared in the same regions in which it was found in 1921, that is, in Florida and Georgia. It later spread practically throughout the flue-cured tobacco areas of Georgia, North Carolina, and Virginia, and into the air-cured tobacco region of southern Maryland. It was also found for the first and only time in Louisiana, in St. James Parish (Fig. 1). Not only was the distribution of the disease very much wider in 1931 than ten years earlier, but it was very abundant in many of the infected areas. By June, half of the tobacco plant beds in Johnston, Wayne, and Wilson counties of North Carolina were infected; moreover, in many beds a large part of the plants were killed. In the aggregate, however, commercial damage was much less than anticipated since injury was confined to the seed beds which many growers habitually plant in excess of their needs.

There were occasional reports of its occurrence during the unusually warm winter of 1931-32. On December 30, 1931, twenty per cent of the plants in a seed bed at Tifton, Georgia, showed some infection. On January 21, 1932, traces of the disease were seen on seedlings in a bed of volunteer plants in Tift County, Georgia. In February, it was reported from Florida, and during the season of 1932 downy mildew extended its range throughout the flue-cured regions of Florida, Georgia, South Carolina, North Carolina, and Virginia; spread to the burley area of central Virginia; recurred in southern Maryland; and was found in Lancaster County, Pennsylvania (Fig. 2). Losses in some of these states, notably the Carolinas, were very heavy in 1932.

Comparison of Fig. 3 with Fig. 2 shows the degree to which the range of the disease was extended in 1933. It was again general in Florida, Georgia, South Carolina, and in the previously affected regions of central Virginia and southern Maryland. It was also found again in Lancaster County, Pennsylvania. During 1933 it spread further in the burley area of central Virginia, and appeared for the first time in the burley areas of southwestern Virginia,

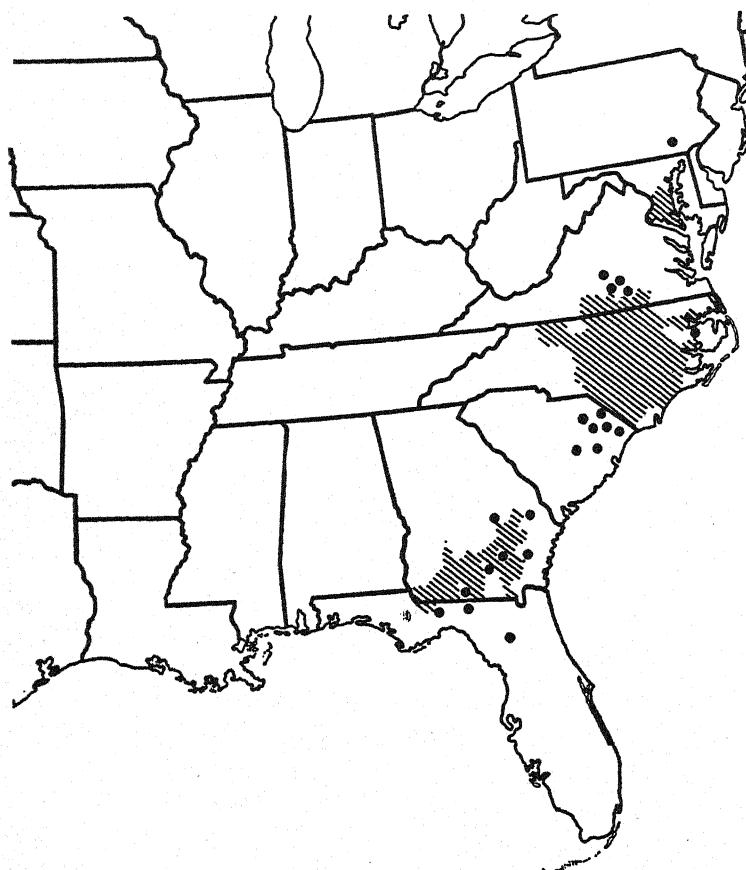


FIG. 2. Tobacco downy mildew in 1932. Shaded portion indicates region affected in 1932 and the previous year. Dots indicate location of counties in which the disease was first found in 1932.

western North Carolina, and eastern and middle Tennessee. That this was actually the first appearance of the disease west of the mountainous regions of western Virginia and North Carolina and eastern Tennessee, and not merely the first report of a disease present in previous years, seems highly probable. One has only to witness the consternation occasioned in an entire community by the general appearance of downy mildew to realize the extent to which tobacco beds are a matter of constant, usually daily, interest to their owners. In such a crop, so conspicuous a disease is not likely to

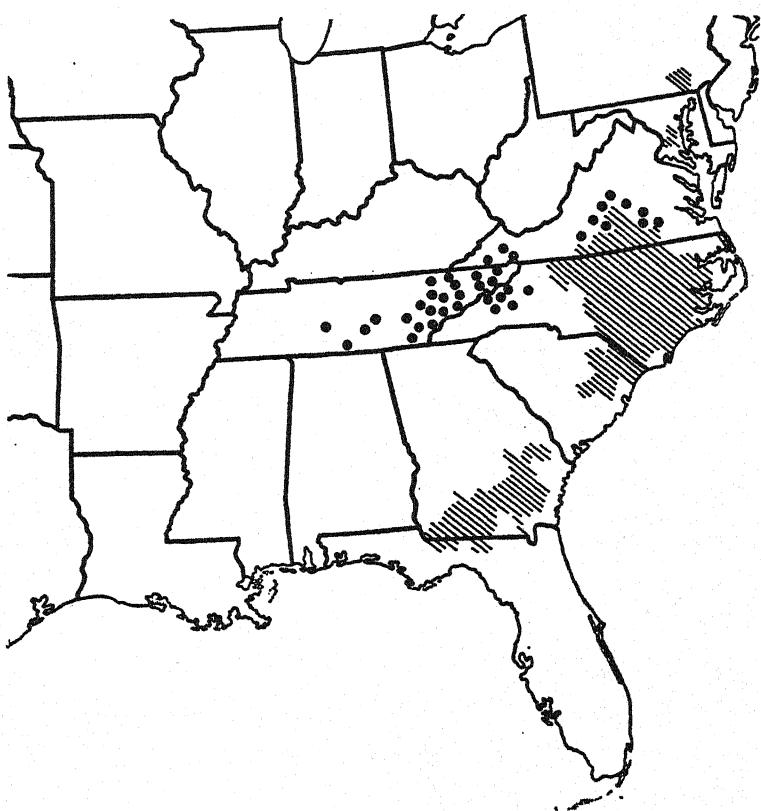


FIG. 3. Tobacco downy mildew in 1933. Shaded portion indicates region affected in 1933 and previous years. Dots show location of counties in which the disease was first found in 1933.

have been overlooked, especially after the publicity given to the 1931 outbreak.

Certainly as regards extent of known range, 1933 appears up to the present time to have been the "climax" year for downy mildew of tobacco in the United States, as it was for bacterial wilt of maize, which is discussed below. The maps show only counties that are specifically named in reports of downy mildew. They are all counties in which tobacco is an important crop. Probably at its peak in 1933, downy mildew actually occurred to some extent throughout the southeastern states wherever tobacco was grown. Partly because of a great increase in seed bed areas and better understanding of the whole situation by growers, actual commercial

losses in 1933 were less than in 1932, but there is nothing to indicate that the disease itself was less serious.

If downy mildew was present in the mountain region in 1934 and 1935, it did so little damage that it was not noticed. In both years it was reported as "general" in Florida, Georgia, South Carolina, North Carolina, Virginia, southern Maryland, and also occurred again in Lancaster County, Pennsylvania. In 1935 it was found in two new counties in Tennessee—Smith and Wilson (Fig. 4). Apparently, the disease was generally less severe and commercial damage was smaller in 1934 than in 1935, but throughout the area it was less severe in both 1934 and 1935 than in 1933.

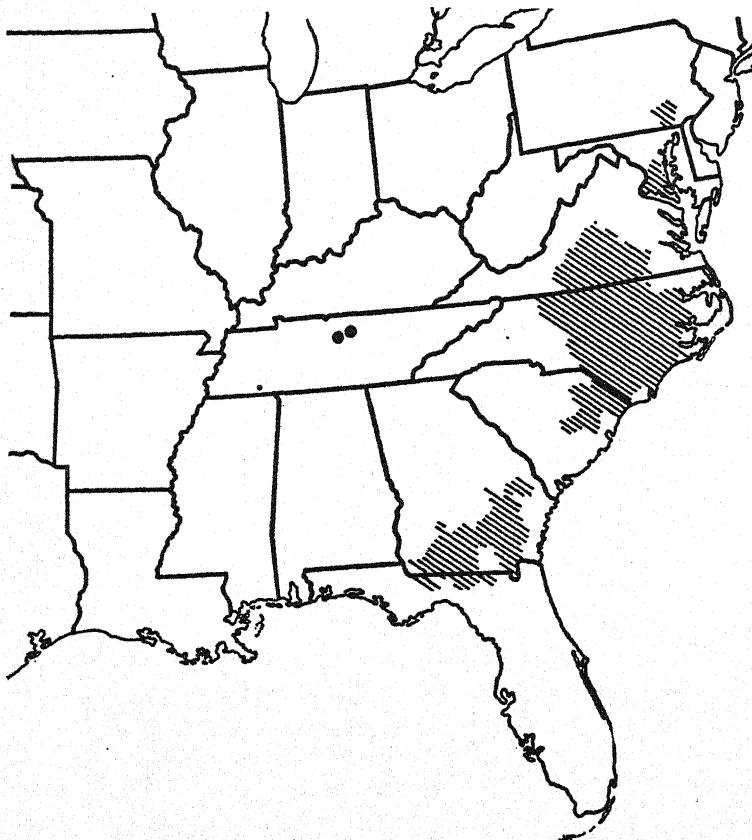


FIG. 4. Tobacco downy mildew in 1934 and 1935. Shaded part indicates region affected in both years. Dots show location of counties in which disease was first found in 1935.

BACTERIAL WILT OF MAIZE

Bacterial wilt of maize (*Zea mays L.*), caused by *Aplanobacter stewarti* (EFS) McCul., was discovered on Long Island, New York, in 1894. Up to 1931 it had been reported from twenty states, most of them south of the latitude of Long Island. Long before 1930, however, the varieties grown in different regions had been well adjusted to the known range of the disease. South of the Potomac and Ohio Rivers the flint varieties and the yellow sweet varieties, on which alone the disease is usually commercially serious, were rarely grown. North of this region the disease was of only occasional importance. The status of the disease was accurately summarized by Chupp in 1925 (2: 123) as follows: "the total amount of injury in the whole country is very slight."

Beginning in 1931 there occurred in the northeastern United States what was apparently the most notable fluctuation in the history of bacterial wilt and one of the most striking fluctuations of any disease in the period under review. Although there were noted during this period occasional cases of severe loss in field varieties of maize, the reports and estimates for the various years relate almost exclusively to "sweet corn," which designation will be used throughout the following summary. In 1931 appreciable losses

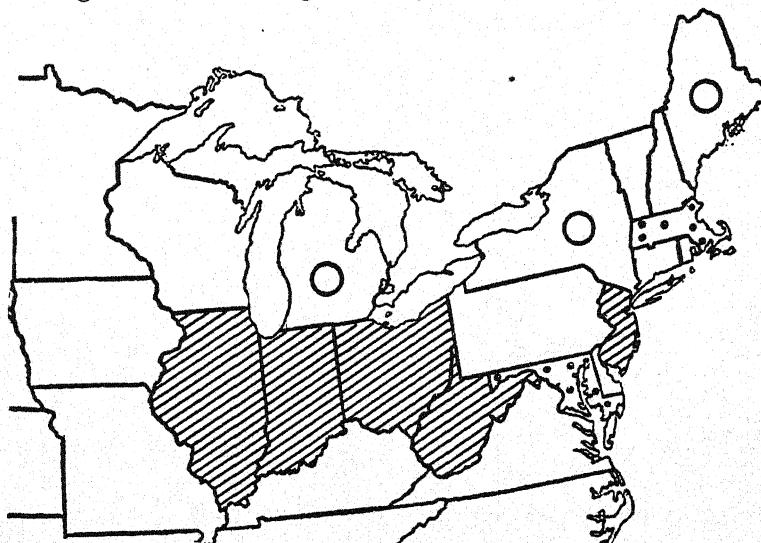


FIG. 5. Loss from bacterial wilt of sweet corn in 1931. Shading, loss appreciable, more than a trace. Dots, loss a trace. Circles, no loss.

from bacterial wilt on sweet corn were observed in New Jersey, West Virginia, Ohio, Indiana, and Illinois. All other states from which reports were received indicated no loss or merely a trace (Fig. 5).

The following year, 1932, there was a marked increase in the range of the disease and in its severity over most of the area where it caused appreciable losses in 1931 (Fig. 6). Neither this map

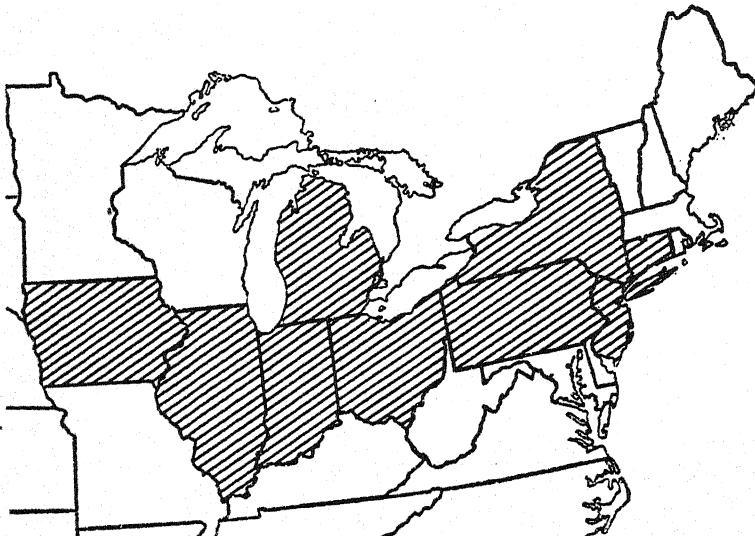


FIG. 6. Loss from bacterial wilt of sweet corn in 1932 compared with 1931. Shaded portion indicates states estimating a marked increase (5 per cent or more) in loss from bacterial wilt in 1932 over 1931.

nor the curve of estimated losses for the whole area (Fig. 9) gives anything like an adequate picture of the destruction caused by the disease on early plantings of the susceptible varieties of sweet corn so generally grown for market. Reports of individual fields with from 30 to 80 per cent of the plants killed were common, and Zundel referred to its results in Pennsylvania as a "massacre rather than a heavy infestation." Particularly notable was the heavy loss in Connecticut—almost certainly the first of such magnitude since the discovery of the disease.

In 1933 the northward range was further extended, and commercial losses were reported for the first time in Maine and Massachusetts. Heavy losses were reported again in the market garden

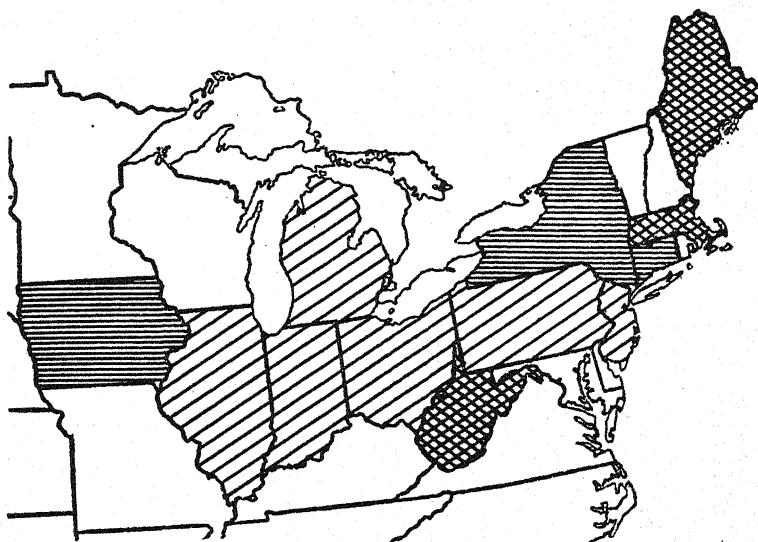


FIG. 7. Loss from bacterial wilt of sweet corn in 1933 compared with 1932. Cross hatching, marked increase (5 per cent or more) in loss over 1932. Horizontal lines, loss approximately the same as in 1932. Diagonal lines, loss severe but decidedly smaller than in 1932.

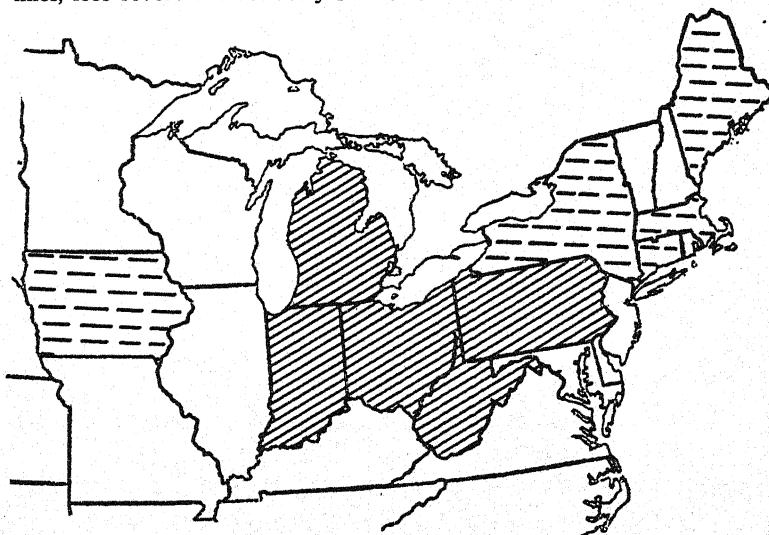


FIG. 8. Loss from bacterial wilt of sweet corn in 1934 compared with 1933. Diagonal lines, loss decidedly smaller than in 1933 but disease still of commercial importance. Broken lines, decrease so marked that disease was of little or no commercial importance.

regions of New York and Connecticut. In New Jersey and Pennsylvania, however, as well as in the North Central states, the losses, while still heavy, were less than in 1932. It was the general opinion of informed observers, however, that this reduction was due more to the elimination of the most susceptible varieties than to a decrease in the abundance of the causal agent.

The situation in 1934 was in striking contrast to that of the two preceding years. As shown in Fig. 8, the disease ceased to be of commercial importance in New York and New England. The states further south and west reported a decided lessening in the severity of the disease. In 1935 there was a further decrease (Fig. 9).

THE CROP LOSS ESTIMATES COMPILED BY THE PLANT DISEASE SURVEY

Since its formal organization in 1917, the Plant Disease Survey of the United States Department of Agriculture has regularly compiled estimates of losses due to disease in certain of the more important agricultural crops. Its plan of operation is to assemble from pathologists throughout the United States, estimates in per cent of losses due to various diseases within their localities. The data obtained from all these sources are combined and preliminary estimates are sent to collaborators and to pathologists of the Bureau of Plant Industry for their criticism and final revision.

In these tabulations, losses are expressed in percentages of the total crop and in amount (in commercial units) of reduction in yield due to diseases. Reduction in yield is calculated by considering the production for the year as 100 per cent minus the percentage of loss from disease. The average estimated percentage of loss for the United States is obtained by dividing the total reduction due to all diseases by the sum of the known production plus the estimated reduction. Since reports are rarely received from all the states in which a crop is grown, the estimates are, strictly speaking, not for the country as a whole but for a "reporting area" which differs with different crops.

Aside from this incompleteness, the principal source of error in the final figures is the inevitable inaccuracy of the estimates submitted by the collaborators. That these are, at best, merely the opinions of well informed and experienced men is evident, and that, at worst, they include many figures which are little more than

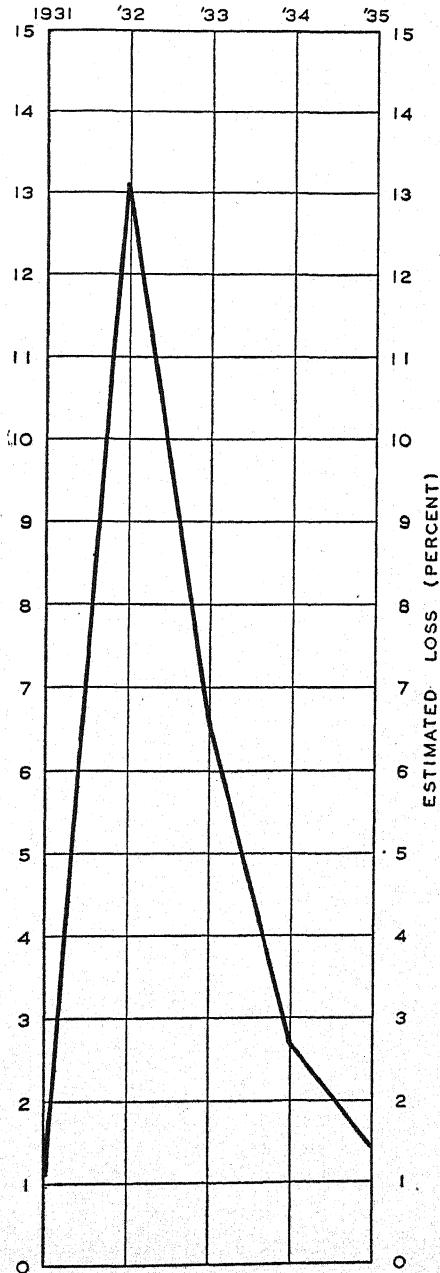


FIG. 9. Losses from bacterial wilt of sweet corn in the United States (reporting area), 1931 to 1935.

guesses is admitted. In the few cases where it has been possible to compare these estimates with figures from wholly independent sources (11, 15), they have appeared to be highly significant, a fact which has greatly increased confidence in the Plant Disease Survey estimates. In this paper are included only estimates which have been so compared or which are the results of special studies which give them unusual authority.

A graphic summary showing the fluctuations in losses caused by certain diseases during the period 1925 to 1934 was included in the 1934 estimates of crop losses issued by the Plant Disease Survey (17: 3-13). Some of these diseases are discussed in this paper; the others are leaf rust and bunt of wheat; stem rust and crown rust and smuts of oats; smut and root rot of corn; potato late blight, leaf roll, mosaic, and rhizoctonia; sweet potato stem rot and black rot; bitter rot, black rot, fire blight, and rust of apple; and peach leaf curl. It has not yet been possible to compare these estimates with figures from other sources, but it is probable that in many cases the relative amounts and the directions of the fluctuations shown in the graphs are as real as those already established through such a comparison. The variations are much smaller than most of those discussed in this paper, but there is no reason to suppose that under favorable conditions some of these diseases would not show as sudden marked increases and decreases in amount. As a matter of fact, such changes have occurred locally in some cases, as for instance, wheat bunt in Pennsylvania and Kansas and other eastern and middle-western states, where there was a sudden sharp increase in loss about 1924, followed by a more gradual reduction, apparently due to the adoption of control measures.

These estimates and, indeed, current opinions regarding crop losses from plant diseases, of which they are perhaps only a concrete expression, are probably much too low. In general, an intensive study of the disease losses of any crop leads to the conclusion that they are much greater than the Survey estimates, a conclusion which is sometimes followed by the assumption that the losses in this crop are particularly heavy. For such a summary as the present, however, which is concerned with the fluctuations in disease, it is not important that the estimates be accurate but merely that from year to year they be reasonably comparable.

"Reporting area" rather than the country as a whole is specified because all states from which no estimates of loss for any one crop are received are left out of the calculations for that year. Since the purpose of the estimates is to obtain a figure as representative as possible of the total percentage loss in the country as a whole, the reporting area for any disease each year comprises all the states that have sent any estimates for a particular crop, whether those estimates include one for the disease under consideration or not. Among these there are often some states in which the disease causes little or no loss or in which it is not known to occur. The reporting area varies somewhat from one year to another, since some states send estimates every year while others report irregularly. Over a period of years, however, it will include, usually, practically all of the states in which a crop is important. The reporting area thus relates to the host rather than to the disease. The only exception is in the case of sweet potato storage rots, in which the reporting area is for the disease itself. Since the loss here is to the crop already harvested, the storage rots estimates are not added to those of other diseases of sweet potato but are calculated separately, directly from the production figures.

LOSSES FROM BACTERIAL WILT IN SWEET CORN

The curve of losses in sweet corn from bacterial wilt, 1931 to 1935 (Fig. 9), is based on the reports of collaborators to the Plant Disease Survey, just discussed.¹ Of its essential significance there can be no question. The disease was the object of much interest and special study during this period, and the maps, Figs. 4, 5, and 6, indicate that the extraordinary losses recorded for 1932 and 1933 were the result of severe incidence of the disease in areas where it had been scarce or unknown in previous years and where susceptible varieties of sweet corn were frequently grown. That the disease declined sharply in 1934 and 1935 throughout this same area is attested by published reports from numerous experienced observers.

LOSSES DUE TO CORN EAR ROTs

The significance of the estimates of losses from corn ear rots, due to various fungi, is the most fully attested of any thus far accumu-

¹ The reporting area for bacterial wilt included Massachusetts, Maryland, West Virginia, Indiana, and Michigan for the whole period and Maine, Connecticut, New York, New Jersey, Pennsylvania, Virginia, Tennessee, Ohio, Illinois, Wisconsin, Minnesota, Iowa, Missouri, North Dakota, Kansas, and Texas for one or more years.

lated by the Plant Disease Survey (15). In Figure 10 the estimated losses due to ear rots compiled from reports of collaborators to the Plant Disease Survey² are compared directly with information derived from a wholly independent and quite different source, namely, the reports of federal grain inspectors of the Bureau of Agricultural Economics. The curve of percentage of cars showing more than 5 per cent damaged kernels at terminal markets is derived from an almost wholly objective measure based on a large number of inspections by skilled and experienced observers. The essential

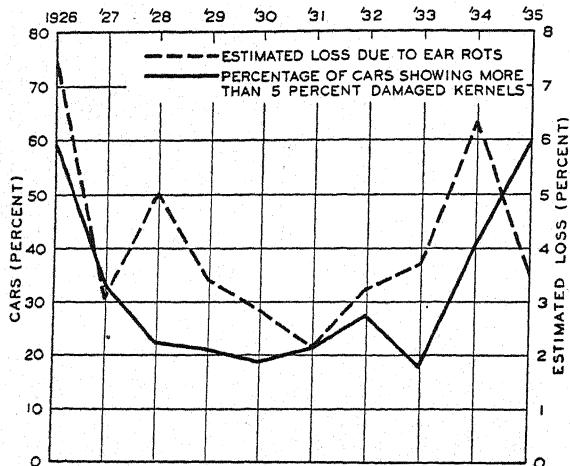


FIG. 10. Percentage of cars of corn showing over 5 per cent damaged kernels as indicated by reports of Federal grain inspectors of the Bureau of Agricultural Economics for the years 1926 to 1935, inclusive; and estimated losses in corn due to ear rots for the United States (reporting area) compiled from reports of collaborators to the Plant Disease Survey, Bureau of Plant Industry.

agreement of the two graphs in so many years can leave little question that they represent comparative conditions with accuracy, and that the losses in the crop of 1926 were actually higher than in any other year during the period. That the divergence between the two curves in 1935 represents an actual difference between the amounts

² States reporting corn ear rots for all 10 years: Massachusetts, Maryland, West Virginia, North Carolina, Ohio, Wisconsin, Minnesota, Kansas, Texas. Reporting for 5 years or more: Connecticut, New York, Pennsylvania, Delaware, Virginia, South Carolina, Georgia, Florida, Indiana, Illinois, Michigan, Iowa, Missouri, North Dakota, South Dakota, Nebraska, Louisiana, Arkansas, Montana, Colorado, Oregon. Reporting for less than 5 years: Maine, New Jersey, Tennessee, Alabama, Mississippi, Arizona, Utah, Idaho, Wyoming, Washington, California.

of ear rots at harvest time and near the end of the storage period, is attested by the fact that Dr. M. T. Jenkins and his associates found that corn ear rots were very scarce at the time of the 1935 harvest on uniform Krug top-crosses in various corn belt states. The fluctuation in the amount of ear rots indicated by the curves is almost certainly the result of actual variation in the incidence of the diseases concerned and not the result of differences in the application of control measures. The methods used for the reduction of ear rots—crop rotation and variety selection—are generally recognized, and change in their use during the period under consideration has been gradual.

Further evidence of the significance of this curve is found in what is perhaps the most accurate of all published records of fluctuations in the severity of disease in a single crop in one locality over a period of years—the report of Benjamin Koehler (7:53-56) regarding the incidence of ear rots in a six-acre plot of two types of open-pollinated corn on the Illinois Experiment Station Farm, Urbana, 1924-35. In Fig. 11 the data are given in per cent of

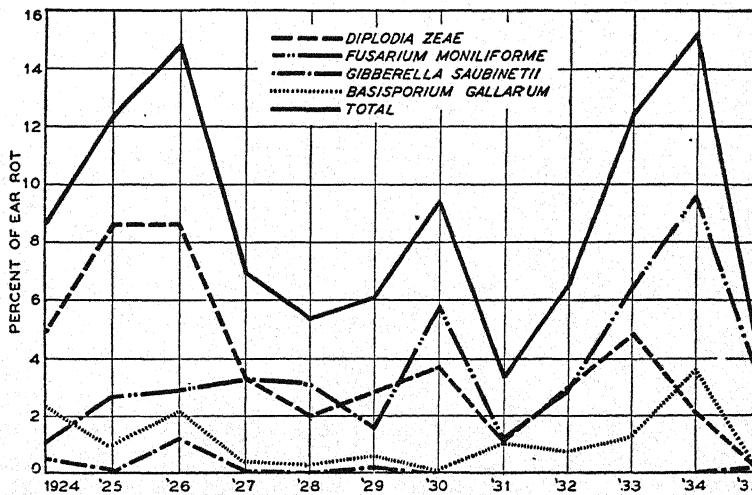


FIG. 11. Relative incidence of ear rots in a six-acre plot of two types of open pollinated corn at Urbana, Illinois, 1924-1935. (After Koehler (7:54, fig. 11).)

actual weights of rotted ears classified at time of harvest. When it is remembered that Koehler's figures are based on conditions in a single locality and those published by the Plant Disease Survey are

averages of estimates throughout the reporting area, including, of course, Illinois, the resemblance is striking. Both curves range through the same degree of amplitude, *i.e.*, relative distance from high to low point. In both curves the 1931 crop shows the smallest amount, and the 1926 and 1934 crops the largest amounts, of rot.

Koehler's figure also gives rather detailed information as to the extent to which the various ear rot fungi caused decay in the different crops, which may be compared with somewhat similar material compiled by Hoppe and Holbert (5) in an extensive study continued over three years in which numerous cultures were made from samples of damaged corn received from various terminal markets. These investigators have shown that fungi causing ear rots of corn vary not only in total damage caused but in relative abundance from year to year. In Indiana (Fig. 12), for example, which may be taken as fairly typical of the corn belt, *Diplodia* was

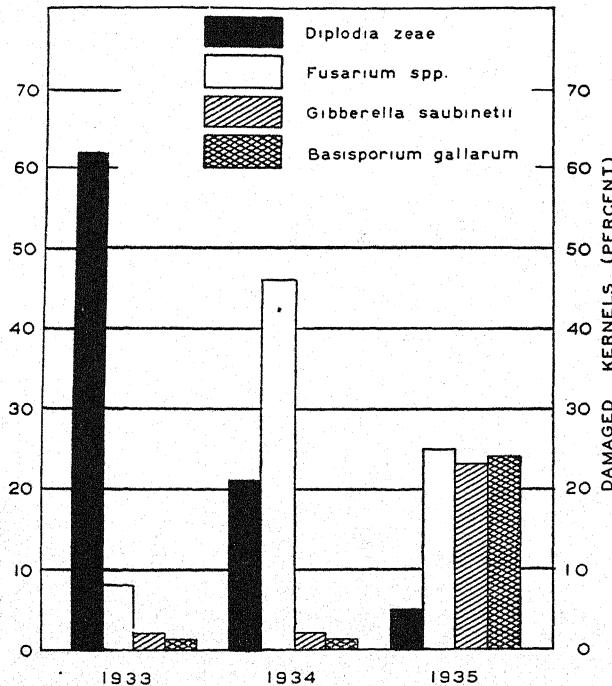


FIG. 12. Percentages of different fungi isolated from damaged kernels in samples from carload lots of corn received at Indiana terminal markets 1933, 1934, and 1935.

the most important cause of rot in 1933, and *Fusarium* in 1934, while three were nearly equal in 1935. In comparing Figures 11 and 12, it should be borne in mind that Koehler's figures relate to conditions in a single field at harvest time and Hoppe and Holbert's to those in a number of terminal markets in June.

There must be many other crops in which, in addition to the major fluctuations in disease losses which may be observed with relative ease, there are variations which can be detected only by means of special investigations. In this category should probably be included those cases in which a particular type of loss may be caused by any one of a relatively large number of organisms, such as the storage rots of fruits, vegetables, or grains. In a series of studies carried out from 1916 to 1918, Stevens and Bain (14) showed that the storage rots of cranberries differ not only in amount but in kind from year to year. That is, the fungi most active as agents of decay one year may be exceeded in importance by others the following season, a fact which sometimes greatly complicates the matter of control.

LOSSES FROM STEM RUST OF WHEAT

The losses from stem rust (caused by *Puccinia graminis* Pers.) of wheat (*Triticum aestivum* L.) in 1935 far exceeded those of any year since the formal organization of the Plant Disease Survey in 1917 (Fig. 13). The highest average estimated loss hitherto reported to the survey was in 1920, only a little over 6.3 per cent. The epidemic of 1935 was one of the most destructive in the history of wheat growing in the United States.

The average loss in 1935 (29.4 per cent) for the barberry eradication states, of course gives an inadequate picture of the actual losses suffered in the more severely affected areas. In Minnesota, where wheat rusts have been studied intensively for many years, the loss was estimated as 56 per cent, North Dakota, 57 per cent, and South Dakota, 29 per cent. Johnston, *et al.*, (6: 19) say regarding Kansas, "The epidemic of 1935 was beyond question the most severe since 1904." Atkins (1: 31) states, "The 1935 epidemic was one of the most destructive in history and extended over most of the wheat-growing areas of the United States," and Waldron (16: 3) concludes, "In fifty years of North Dakota farming, there have been only three first-class epidemics—these were 1904, 1916, and 1935."

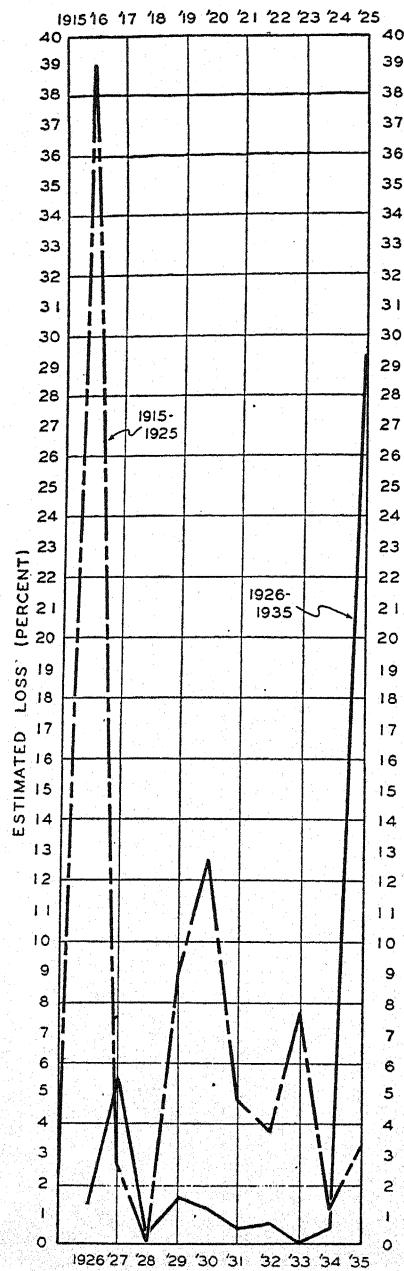


FIG. 13. Estimated percentage losses from stem rust of wheat in the thirteen states in the barberry eradication area, 1915-1925 (broken line), and 1926-1935 (solid line).

Since the records of the Plant Disease Survey do not include the year 1916, we have placed in Fig. 13 for comparison, a curve for the years 1915 to 1924 based on figures given by Heald (4: 778) of estimated losses from stem rust of wheat in the 13 barberry-eradication states (Colorado, Indiana, Illinois, Iowa, Michigan, Minnesota, Montana, Nebraska, North Dakota, Ohio, South Dakota, Wisconsin, and Wyoming), and have included in the curve for the years 1925-1935 only estimates of barberry eradication agents and reports of collaborators to the Plant Disease Survey from the same states. While the two curves are thus not based on exactly the same material, it is apparent from study of the figures for the years 1917 to 1924 derived from both sources that they are reasonably comparable.

STORAGE ROTS OF SWEET POTATO

The sweet potato (*Ipomoea batatas* Lam.) is one of the few crops in which losses in storage, caused by various fungi, have been reported in sufficient detail to warrant compilation (Fig. 14).³ In his earlier summary, Stevens (11: 981) suggested that the sharp decline in estimated losses from the high point in 1918 to a low which was maintained from 1922 to 1926, might be due to the effectiveness of an intensive campaign for their reduction led by L. L. Harter and F. C. Meier, and that the somewhat higher losses indicated for 1927, 1928 and 1929 might be correlated with the lessened interest in this work. The somewhat higher average estimated losses since 1929 and in particular the more marked and irregular fluctuations in amount of loss from storage rots during this period seem to strengthen the probability of the correctness of this view.

PEACH BROWN ROT

The significance of the curve of estimated losses due to brown rot, caused by *Sclerotinia fructicola* (Wint.) Rehm, of peach

³ States reporting sweet potato storage rots for all 9 years 1917-1925: Virginia, South Carolina, Georgia, Kansas, Tennessee, Mississippi; reporting for 5 years or more: New Jersey, Delaware, Maryland, North Carolina, Indiana, Illinois, Kentucky, Alabama, Texas, Oklahoma, Arkansas; reporting for less than 5 years: Pennsylvania, West Virginia, Florida, Ohio, Iowa, Missouri, New Mexico, California, Arizona.

States reporting for all 10 years 1926-1935: Maryland, North Carolina, South Carolina, Arkansas; reporting for 5 years or more: Delaware, Virginia, Tennessee, Georgia, Florida, Texas, Iowa, Kansas; reporting for less than 5 years: Pennsylvania, New Jersey, Alabama, Mississippi, Louisiana, Indiana, Missouri, Oklahoma, California.

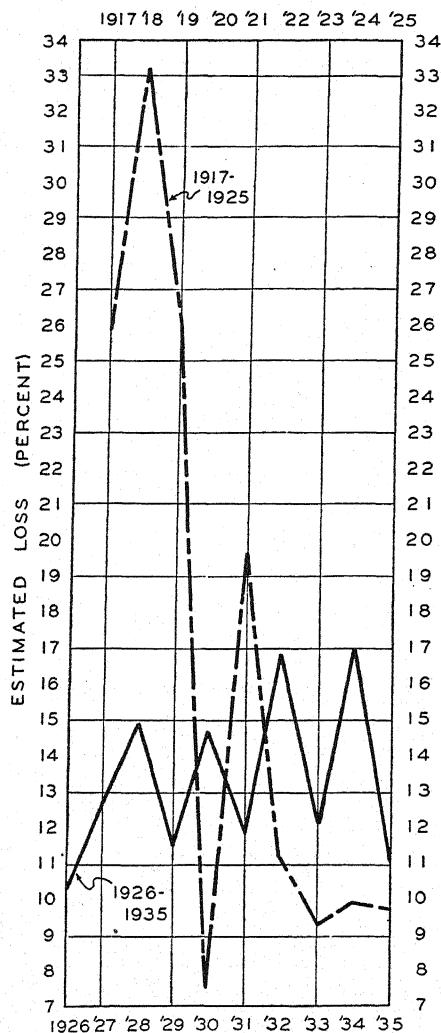


FIG. 14. Estimated percentage losses from storage rots of sweet potatoes in the United States (reporting area), 1917-1925 (broken line), and 1926-1935 (solid line).

(*Amygdalus persica* L.) for the years 1922 to 1928, was discussed in detail by Stevens (11: 980). For this period the estimates of Plant Disease Survey collaborators agreed very well with information derived from a wholly independent source, namely, the relative

abundance of brown rot of peaches at terminal markets as shown by reports of the Food Products Inspection Service. There is no reason for considering the subsequent estimates less reliable, and the figures for the years 1926 to 1935 are given in Figure 15.⁴

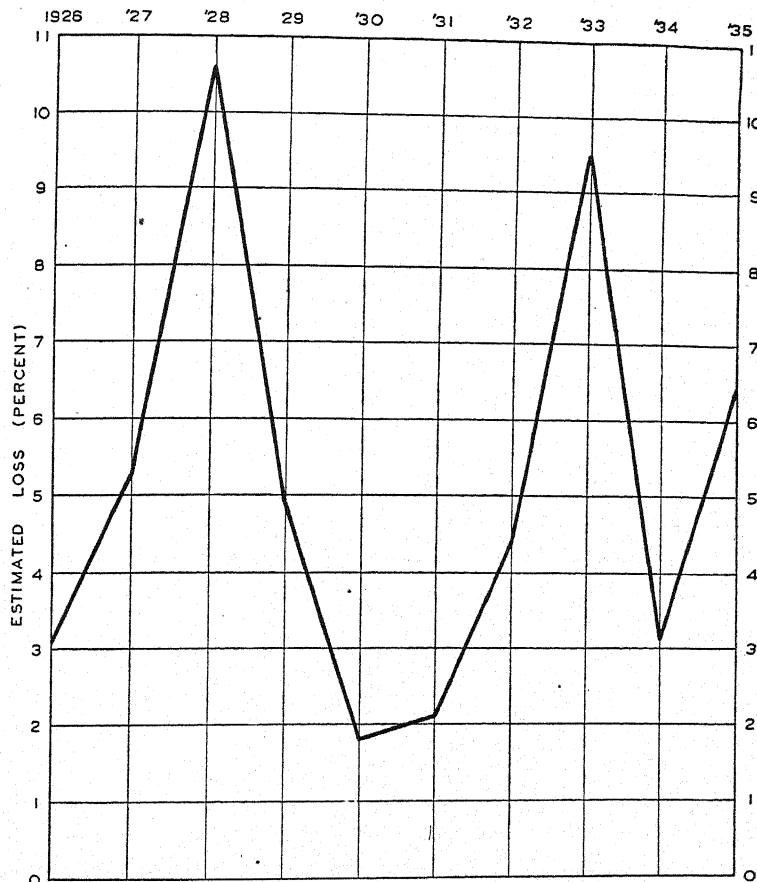


FIG. 15. Estimated percentage losses from peach brown rot in the United States (reporting area), 1926-1935.

⁴ For peach brown rot the reporting area is as follows: for all 10 years: Maryland, North Carolina, Michigan, Arkansas; for 5 years or more: Massachusetts, Connecticut, New York, New Jersey, Pennsylvania, Delaware, Virginia, West Virginia, South Carolina, Georgia, Florida, Kentucky, Tennessee, Mississippi, Ohio, Indiana, Illinois, Iowa, Missouri, Texas, Washington, Oregon; for less than 5 years: New Hampshire, Rhode Island, Nebraska, Kansas, Alabama, Louisiana, Oklahoma, Arizona, Idaho, Colorado.

APPLE SCAB

The correctness of the estimates of losses due to scab, caused by *Venturia inaequalis* (Cke.) Wint. of apple (*Malus sylvestris* Mill.), is as yet unsupported by evidence derived from a source other than the judgment of collaborators. The disease is so generally known and so intensively studied, however, that it has seemed worth while to include estimates for the past decade (Fig. 16), especially since the reporting area for apple scab includes, in one year or another, practically every state in which the crop is of commercial importance. The estimated loss from apple scab in 1935 is the highest since the establishment of the Plant Disease Survey. Unlike some of the diseases already discussed for which there are at present no practicable means of field control, apple scab is largely controllable by methods long generally known. This is also true of peach brown rot. Indeed, variation in the thoroughness of the application of control measures has been offered as at least a partial explanation of the fluctuation in the severity of peach brown rot between 1920 and 1929 (11: 980). It is possible that this may have been of local importance in increasing the abundance of apple scab in 1935; that is, some growers were led to neglect spraying somewhat that year as a result of the extreme scarcity of the disease in 1934. This can hardly have been a major factor, however, since more general and intensive organized effort is regularly given to the control of scab in important apple-growing states than to any other disease.

CURLY TOP OF SUGAR BEETS

So many factors other than disease ordinarily enter into total yield that only rarely can yield be related directly to disease losses. Those engaged in the study of cranberry diseases, are, however, apparently in agreement that the continued decline in cranberry production in New Jersey since 1923 is largely due to the inroads of the false blossom disease (11: 984. Fig. 9). This change can hardly be classed as a fluctuation and is thus outside the scope of the present paper.

Marked and rapid fluctuations apparently due chiefly to disease are shown in the yield of sugar-beets in certain areas. The per acre yields of sugar beets in Idaho during the period 1920-1934, as compiled from the U. S. D. A. Yearbooks by Hartley and Rathbun-Gravatt (3: 165), Fig. 17, show a change from 7 tons per acre in

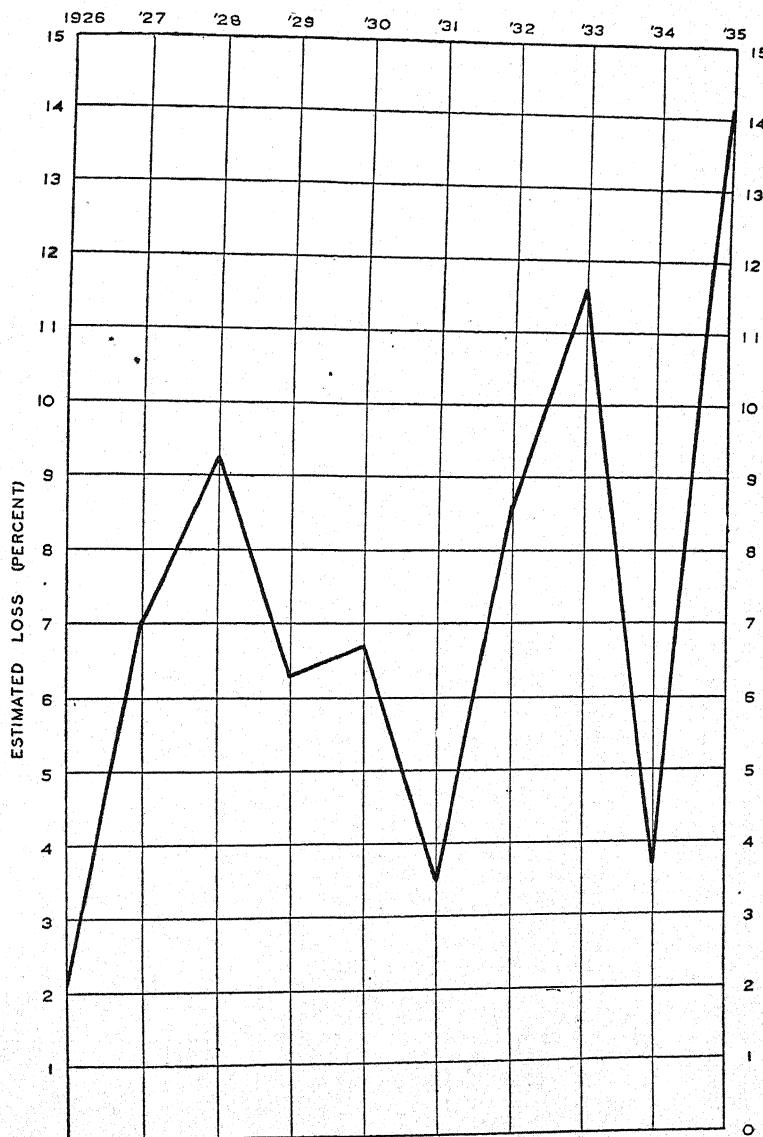


FIG. 16. Estimated percentage losses from apple scab in the United States (reporting area), 1926-1935.

1924 to almost 13 in 1925, down to 6 in 1926 and up to over 13 in 1927, and somewhat smaller differences during the remainder of the period. These changes are in the opinion of those best informed, due chiefly to differences in the severity of curly top. Confirmation of this is found in the relative constancy of yield in Colorado where about 90 per cent of the crop is grown outside the curly top areas (Fig. 17).

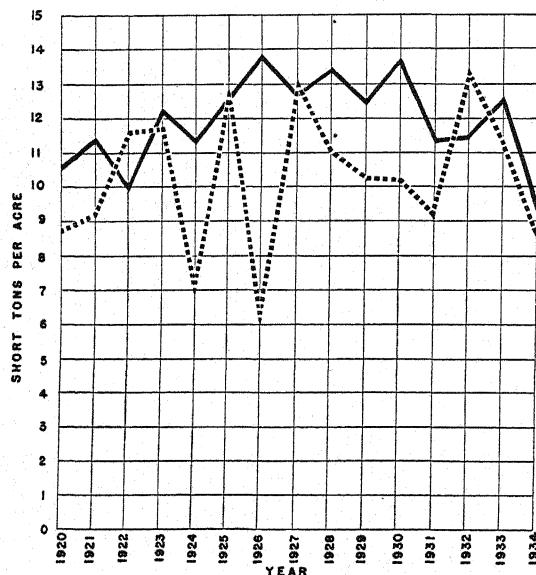


FIG. 17. Sugar-beet yields in Idaho and Colorado. Solid line, Colorado where 90 per cent of crop is grown outside the curly-top areas. Broken line, Idaho where curly top is prevalent. After Hartley and Rathbun-Gravatt (3: 165, Fig. 3).

LOSSES FROM CRANBERRY FRUIT ROTs

The relatively unimportant cranberry (*Vaccinium macrocarpon* Ait.) is included in this review because, as a result of the continued studies of C. L. Shear and his associates, there are available more accurate data on the losses in cranberries, due to fruit rots, than for any other fruit. Indeed, the very accuracy and completeness of this information has given rise in certain quarters to the apparently erroneous impression that losses in cranberries are much larger than in other crops. Fig. 18, which gives the relative incidence of cranberry fruit rots in Massachusetts, is based on the results of storage

tests of eight to ten lots of the "Early Black," the most important variety in that state (13). These storage tests have been continued for a number of years, and the lots so tested are taken from the same bogs in the Wareham-Carver area year after year.

WHAT IS MEANT BY "GOOD" KEEPING QUALITY

In comment on the graph of cranberry rots, it should be pointed out that those commercially interested in Massachusetts cranberries considered the crops of 1931 and 1933 as of very poor keeping quality, the crop of 1932 very good, and the crops of the two other years as good—but not exceptional. From a comparison of actual losses of the lots held in storage it is apparent that the margin between "good" and "poor" crops may be very narrow. There is always some rot. On the other hand, during even the worst years a majority of the berries are sound at marketing time. The condition here is somewhat comparable to atmospheric or soil temperatures; a difference of 2 or 3 degrees in average daily temperature means a great difference in the weather for the year. What actually happens, of course, in years of poor keeping quality is that rot develops to a dangerous extent in some lots of fruit that are usually sound.

"Good" and "poor" keeping quality, as used in discussions of cranberry problems are, of course, relative terms and vary with the variety under discussion and with the locality in which it is grown. The keeping quality of berries is judged inevitably in terms of commercial practice. If the fruit of a given year undergoes successfully what is usually demanded of it, the quality is said to be "good"; otherwise, it is called "poor." It is probably unnecessary to add that this same subjective criterion is the one actually applied to any other fruit or grain crop, just as it is the one used in judging human achievement.

ATTEMPTS TO APPRAISE THE ECONOMIC EFFECTS OF FLUCTUATIONS IN PLANT DISEASES

In many papers on plant pathology issued a decade or two ago, there is included a statement, in dollars, of the economic losses due to certain plant diseases. The often startling figures so published were obtained usually by multiplying the commercial units estimated as lost by the price received for the portion of the crop actually sold.

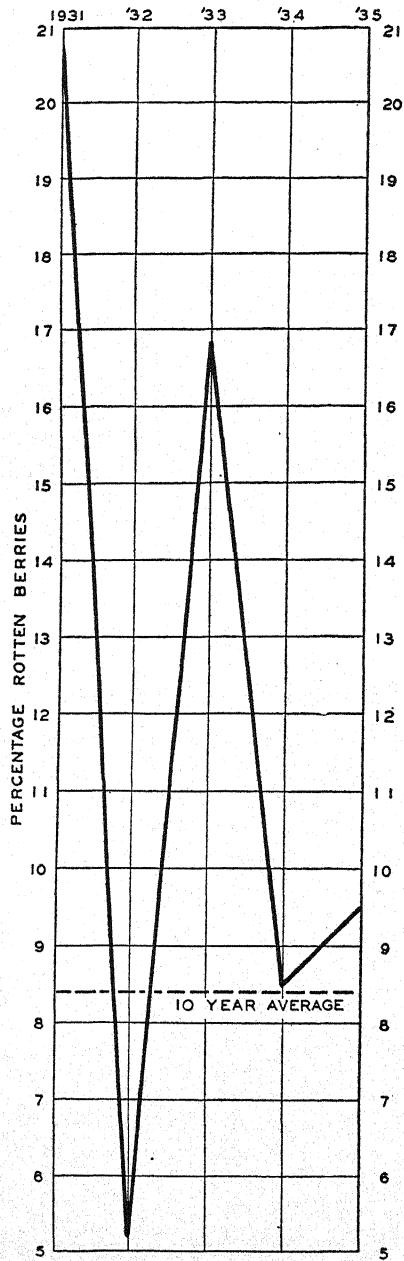


FIG. 18. Relative incidence of cranberry fruit rots in Massachusetts, 1931 to 1935, as indicated by the average percentage of rotten berries in storage lots of the Early Black variety on October 15.

Obviously, in a country severely schooled by national agitation as to some of the hazards of over-production of important crops, such "estimates" were no longer useful, even for propaganda. Recently, a few serious attempts have been made to appraise the actual effects of plant diseases on variability of crop yields as well as the economic effects of variation in yields.

The first of these and the most comprehensive is contained in California Agricultural Experiment Station Bulletin 553 (10), a thoughtful volume of nearly 300 pages, prepared by a committee consisting of two economists, two plant pathologists, and three entomologists, each of recognized standing in his own field, collaborating over a period of three years. By the limitations of the subject assigned them for study, the discussion of "The economic effects of plant quarantine" (pp. 38-81) relates specifically to the effects of introduced pests and of quarantines on the total social income and on the distribution of income among individuals and groups, but their observations are equally relevant to fluctuations in yield due to native pests, or, indeed, to any cause.

These authors point out that the interests of various social groups are not the same in respect to the effects of plant disease. "The individual producer in a competitive society always secures the largest income for himself by obtaining the maximum production possible with the minimum of effort and expense. To him all plant diseases and pests whether controllable or not constitute handicaps which reduce the yield or the quality of his product and increase his costs of production" (p. 42).

The greatest difficulty appears in determining the economic effects of fluctuation due to disease on producers as a group. Various specific examples, too detailed for review, are given of the immediate effects on growers of changes in volume of crop and the complexity of the problem is summarized in the statement: "Sometimes the growers as a group or class might gain, in other cases they would lose—a small change in volume may have one tendency, a large change just the opposite" (p. 51).

The authors find no such conflict of interest in the case of consumers. They say: "In the long run the consumers tend to pay all of the costs and to receive all of the benefits [of pest and disease control measures] except when some form of monopolistic control intervenes, and then consumers continue to pay all of the costs but

do not receive all of the benefits" (p. 44). "The amount growers gain or lose in money is paid or saved by the middleman and the consumer. *The consumer always benefits from an abundance of production and should be more interested in maintaining plant quarantines that insure such abundance than growers as a group*" (p. 51).

"Society considered as a large group of producers and consumers has interests almost identical with those of individual producers" (p. 43).

In discussing the effects of changing quality of products, the writers accept as a working definition of high quality, "any characteristic of a product that commands a premium" (p. 52). They find even greater difficulty in appraising the economic effects of changing quality than of changing quantity, but conclude, "The effects on price and returns to growers from changing the quality of product are very similar to those from changing the volume" (p. 52).

Choosing the cultivated cranberry as a basis of study because it is a highly specialized crop to the diseases of which much study has been devoted and about which very exact information as to price and yield is available, Stevens (12) has attempted an analysis along the lines of the bulletin just discussed. The conclusions reached in this study are largely in line with those in the California bulletin except that he insists that "the cranberry industry appears never to have suffered during the period under review [1912-33 inclusive] from over-production, as such, even in the most limited sense of the word." This is due, in his opinion, to the fact that over-production seems to have been prevented by factors other than disease. Among these are mentioned the large investments necessary in going into the business, the importance of skilled management, and the losses from frost and insects.

Recently, Hartley and Rathbun-Gravatt (3) have attacked the problem of determining the "contribution to yield variation made by plant parasites and the effect that disease-control measures may have on yield variation" (p. 159). Their paper illustrates some of the complexities of the whole problem. The writers point out that most diseases are not independently varying factors. "It is scarcely possible to conceive of a disease whose progress is not influenced by the vigor of the host, or by environmental factors that also affect

the yield in some other way" (p. 160). And in discussing the difficulties which beset the most careful attempts at appraising disease losses, they point out that, "Even where estimates of damage are entirely correct, it is possible, of course, that if the disease had been absent some other factor might have become important in limiting yield. For example, if control of a leaf spot resulted in a 15 per cent greater area of active leaf surface, a marginal soil moisture supply might become submarginal" (p. 162, footnote).

In an analysis of the effect of losses (as estimated) from potato late blight on potato yields in New York for ten years, 1920-1929 inclusive, they conclude that the disease had actually been a stabilizing factor. The authors correctly point out, however, that they are here dealing with a disease the control measures of which are well known and generally practiced, and add that "Before present control procedure was developed and with the varieties that were in use a century ago, the effect of late blight on national yields was anything but stabilizing. One has only to remember its part in causing the historic Irish famine with its resultant loss of many thousands of lives and wholesale emigration to the United States. To put the case in another way, the progress that has been made in the control of the late blight has changed it from a catastrophic thing that upset all expectations, into one which no longer seriously affects the dependability of total regional yields so long as the rather expensive spraying schedules are followed" (p. 165).

These authors, like those of the California bulletin above cited, find that the status of the individual grower may be quite different from a national or even regional group, and that "The variations caused by diseases in the returns to the individual farmer are on the whole more serious from the standpoint of social security than the national or regional yield variations. Farming continues an individual venture, crowded with risks too large for the individual to carry readily. The effect of disease in decreasing crop predictability is much greater for the individual holding or for a local community than for the State or national yields already considered. A disease that has relatively little effect on yield totals may be periodically disastrous to single farms" (p. 168).

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SOME ASPECTS OF THE SALT NUTRITION OF HIGHER PLANTS

D. R. HOAGLAND

University of California

INTRODUCTION

The subject of the salt nutrition of plants is vast in its ramifications and in the extent of its literature. Only a few aspects of this field of investigation can be considered in this brief review, which has as its intent the presentation of the broad outlines of the present status of certain of the basic problems now attracting the active interest of research workers, rather than the comprehensive citation of publications. The review is prepared primarily for botanists without a specialized interest in plant nutrition. For more extensive surveys of recent literature, the reader will be referred to other more detailed reviews.

No other system presented for scientific study by experimental methods is so complex or so difficult to control as the soil-plant system. It is indispensable, for purposes of experimentation planned to elucidate general principles of mineral nutrition of plants, to simplify the natural system. Of paramount importance is the ability to study plant growth without introducing the soil factor at all, through the use of sand and water culture technique. It is now ancient knowledge that plants can be grown in artificial culture solutions containing certain simple salts, but only comparatively recently have several variables and sources of error begun to receive that adequate attention which their importance demands. Growing plants under water or sand culture conditions still affords one of the most potent means of attack on problems of the mineral nutrition of plants, and this review is especially concerned with advances made possible by the utilization of these methods of artificial culture.

CHEMICAL ELEMENTS ESSENTIAL FOR THE GROWTH OF THE HIGHER GREEN PLANT

One question of first importance which has been answered, at least in part, by the results of water and sand culture experiments, is that of the number of chemical elements essential to the growth

of higher plants. A previous review by Brenchley (9) has dealt with this question, but a brief additional discussion seems to be in order, from the point of view of the special problems considered in this review. Early investigations established a list of ten chemical elements essential to plant growth and it became a rather dogmatic teaching of plant physiology that only these ten elements are indispensable. Many other elements were often found in the ash from plant tissues, but the mere presence of an element in a plant, however suggestive, could not be regarded as proof of its essentiality. Neither was such proof afforded by the "stimulation" of growth sometimes observed when minute amounts of certain elements were added to the culture medium. It was necessary to show that plants could not grow in any normal way when deprived of a chemical element in question, regardless of other elements available.

The technique long employed in water and sand culture experiments was too crude to disclose the need for mineral elements effective in minute quantity.* More than twenty years ago, however, Mazé (62, 63, 64) published reports of experiments with maize plants in which specially purified salts and special culture vessels were utilized to exclude impurities. (For reference to other early French work see review by Brenchley (9)). Mazé concluded from these experiments that various chemical elements not included in the list of ten were essential to the growth of plants. Despite certain limitations of the investigation, the results provided definite suggestions for further research. But a long period elapsed before any general recognition was accorded to the importance of elements required in minute quantity for plant growth.

The evidence now available is conclusive that the former list of ten essential elements is incomplete. McHargue (65), by carefully controlled sand culture experiments, verified earlier views that manganese is indispensable to the growth of green plants. Then came the researches of Warington (115), and later of Sommer and Lipman (89), which left no doubt that boron is an absolute requirement for the growth of plants of the species studied. In the past decade, studies on boron have become very numerous, and plants

* Such elements have been termed "rare" elements, "trace" elements, "minor" elements, "cryptotrophic" elements and "micro-elements." Objection can be made to all these terms. Certainly the elements in question are not rare in their occurrence, nor of minor significance for plant growth. The term "micro-elements" is sometimes convenient.

of all the many kinds tested failed to grow normally when the supply of boron was reduced to a sufficiently low value. Doubt was at one time expressed of the need of the barley plant for boron, but Sommer and Lipman demonstrated that when boron was excluded as rigorously as possible by a refined technique, barley plants made almost no growth.

Evidence for the essentiality of copper and zinc is not so extensive as that for manganese and boron, and some investigators are not ready definitely to admit copper and zinc to the group of elements essential for higher plants, yet the experiments of Sommer and Lipman (89), Sommer (90, 91), and Lipman and Mackinney (55) on zinc and copper gave decisive results. The essentiality of zinc was demonstrated in experiments on plants representing five families. Recent work in the laboratory of the reviewer also has shown that maize, apricot and other plants develop symptoms of disease when grown in solutions deficient in zinc, symptoms which are similar to those occurring under certain soil conditions. With reference to copper, positive evidence of essentiality was reported for the vegetative growth of sunflower, flax and tomato (91) and for seed production of barley and flax (55).

The argument is sometimes advanced that the elements under discussion may be essential for some kinds of plants; but not for all. Undoubtedly it is desirable that more kinds of plants be investigated but the complete failure of plants of the diverse types already studied to make normal growth in the absence of an adequate supply of boron, manganese, zinc or copper, surely suggests that the functions of these elements are of a general character. Conceivably a slight reservation should be made concerning copper until the possibility is completely excluded that any other element capable of existing in different valence states can replace copper.

There are suggestions that the addition of boron, manganese, copper and zinc to the list of essential elements leaves this list still incomplete, but the evidence so far presented for the essentiality of other elements effective in minute quantity does not permit conclusions to be drawn with finality. It is interesting to note that Robbins *et al.* (79) observed striking effects on the growth of excised root tips from the addition to the culture solution of the ash of filter paper or of agar, effects which it was thought were probably not attributable to boron, manganese, copper or zinc.

In the experiments which first gave definite evidence of the requirements of plants for boron, copper, manganese or zinc, elaborate precautions were taken to purify the nutrient salts, and to eliminate contamination derived from culture vessels or distilled water. These precautions were indispensable to the achievement of the objective of these experiments, namely, to prove, for every kind of plant studied, a complete dependence of growth or reproduction on the presence of the element under investigation. It might appear, therefore, that the recently recognized essential elements are of slight consequence for general researches on plant nutrition in the greenhouse, conducted without the refinements just mentioned; or for problems of soil and plant interrelations.

The discoveries of recent years make apparent how erroneous such a deduction would be. It has now become a simple procedure to demonstrate, with suitable species of plants, the effects of boron and manganese deficiencies under conditions characteristic of the usual routine of culture solution work. In the experience of the reviewer (46), deficiencies of zinc may also sometimes occur under ordinary water culture conditions, at least when certain types of containers for the culture solution are employed. Furthermore, many investigators in different parts of the world have described nutritional or physiological diseases of plants produced by soil conditions which are cured by applications to the soil, or directly to the plant, of manganese, boron, copper or zinc, according to the nature of the disease (see review by Brenchley) (9). Interest in the effects of these elements is not lessened by the consideration that simple deficiencies in the soil may not always constitute a full explanation of the origin of the diseases. Suggestions have been made concerning the neutralization by metals of toxic organic substances generated in the soil; interference by micro-organisms with the absorption or utilization of zinc or manganese by the plant; oxidation-reduction equilibria in the soil favorably modified by addition of copper, manganese or other metals.

For many years before it was generally recognized that more than ten chemical elements were essential, investigators grew plants more or less successfully by sand or water culture methods, without any deliberate addition to the media of boron, copper, manganese or zinc. This is not difficult to understand in view of the probability that the nutrient salts were often of a low degree of purity and that

the glass or other culture vessels could have contaminated the nutrient solutions. Nevertheless, the question remains if deficiencies of elements regarded at the time as unessential did not sometimes invalidate conclusions concerning the other elements of the culture solution which were under investigation. Johnston (51) cites an interesting experience pertaining to boron which bears on this point.

There is now reason to give heed, in sand or water culture experiments, to the minute constituents of the nutrient solution. It may even be advisable to add to the solution a large number of chemical elements in very small amounts, below the point of toxicity, to avoid any unknown deficiency. Such supplementary additions have been made by various investigators in the form of so-called "A-Z" solutions. Observations on the effects of including in nutrient solutions minute quantities of many chemical elements in different combinations have been recorded by Haas and Reed (36), Schropp and Scharrer (86), Grossenbacher and Livingston (35) and by Hoagland and Snyder (44), among others.

Different kinds of plants seem to have different quantitative requirements for boron, manganese, copper or zinc as well as greatly varying tolerance to the toxic effects which these elements may produce when they are present in the culture medium in concentrations beyond the range of physiological need. Eaton (27) has presented comprehensive data on the relation of boron concentration to the growth of various types of plants, which have special interest, since both boron toxicity and boron deficiency are found under conditions of practical agriculture, and both have assumed considerable economic importance.

FORMS OF NITROGEN IN NUTRIENT SOLUTIONS

Until recent years nitrate was almost always the form of nitrogen utilized in preparing nutrient solutions, although experiments made long ago had indicated that ammonium nitrogen could be substituted for nitrate nitrogen (48, 78). Great interest exists at the present time in the relative efficiency and physiological properties of these two forms of nitrogen and many papers relating to this question have appeared recently. Special attention has been directed to the interrelations between hydrogen ion concentration of the nutrient medium and the absorption or utilization of nitrogen. Various investigators have supported the generalization that nitrate nitrogen

is absorbed most rapidly at a relatively high concentration of hydrogen ion (for example, pH 5) and ammonium nitrogen at less acid reactions, or at alkaline reactions (66, 4, 17, 76, 41, 43, 59, 72, 103). There are, however, several factors which greatly complicate the interpretation of experimental data. It is extremely difficult to ascertain what the effective hydrogen ion concentration is at absorbing root surfaces, particularly in sand cultures. The reaction of a nutrient solution can be changed with great rapidity by the selective absorption of ions and by the metabolically produced CO₂ from the roots. In one recent investigation, consideration of this point has led to modification of conclusions first drawn (109). Another factor sometimes overlooked is the precipitation of calcium, magnesium, phosphate or iron at alkaline reactions, which may make strict comparisons of acid and alkaline nutrient solutions unwarranted.

Recent experiments by Arnon (1, 2, 3) demonstrate that hydrogen ion effects on nitrogen utilization cannot be properly interpreted in terms of a single variable, at least within the most important physiological range of hydrogen ion concentration. Barley plants were grown in large tanks of nutrient solution at different seasons of the year with controlled variations of the following factors: source of nitrogen, nitrate or ammonium; hydrogen ion concentration, aeration, and concentration of certain micro-elements, particularly manganese. Several of the conclusions are of interest at this point: (1) pH effects and climatic environment are interrelated so that generalizations from experiments conducted at only one season are open to doubt. (2) Forced aeration of the nutrient solution produced extremely beneficial effects on the otherwise unfavorable plant growth in the solutions prepared with ammonium as a source of nitrogen. (No nitrate was detected in the solution at any time.) The effects of aeration were slight when nitrate was the source of nitrogen. (3) Manganese or copper added to nutrient solutions containing ammonium salts (over the impurities present), acted to a large degree as a substitute for forced aeration in producing favorable conditions for growth.

The fact has now been established by the results of many investigations that ammonium nitrogen can be used successfully in nutrient solutions, but it appears that the physiological efficiency of this form of nitrogen is more subject to the influence of various factors

of the solution or aerial environment than is nitrate nitrogen. In the soil, because of nitrification, the plant is rarely subjected to a medium in which the sole source of nitrogen is ammonium nitrogen. (An extensive review of this subject was published by Pardo (72).)*

GENERAL NATURE OF PROCESSES OF SALT ABSORPTION BY ROOTS

Before entering on the discussion of quantitative relations of salts in nutrient solutions, it will be convenient to describe some recent developments in knowledge of the general nature of the processes by which mineral solutes are absorbed by roots, which is obviously a question of fundamental importance to plant nutrition in all its aspects. In the past, these processes have usually been entirely misconceived. Even the latest textbooks may fail to note, or to make clear, the revisions of older ideas of salt absorption which are inescapable in the light of evidence now available.

The general problem has long been presented in unambiguous form as a result of studies on the large coenocytic algal cells of different species of *Valonia* (growing in sea-water), *Nitella* (growing in fresh water) and *Chara* (growing in brackish water), from which vacuolar sap can be removed from individual cells with only slight contamination from the cell wall or protoplasm (20, 22, 38, 39, 40, 71, 118). Sap analyses reveal that at some stages of growth these cells possess the power of salt accumulation; that is, the power to absorb certain constituents of the solution bathing the cells, against concentration and activity gradients. *Nitella* and *Chara* cells can accumulate in their vacuolar sap all the principal ions of the system in concentrations higher than those which obtain in the external solution. *Valonia* concentrates potassium primarily. Adsorption on colloids, chemical precipitation in the sap, and Donnan equilibria, fail to explain the accumulation (see previous review by Osterhout (71)). It becomes necessary to postulate that living plant cells can do work in the accumulation of salt with the aid of metabolically derived energy.

The function of salt accumulation is not peculiar to the highly specialized plants just discussed. This function seems to be a general attribute of living plant cells in a state of active growth and

* Since this review was prepared a comprehensive review of nitrogen metabolism of plants has been published in this journal by G. T. Nightingale.

metabolism, and, in fact, mature algal cells do not have the high capacity for further salt accumulation which some other kinds of plant cells possess. The experiments by Steward and his associates on potato and other storage tissues have now led to the development of certain fundamental views of the whole problem of solute accumulation which have a wide application (7: 93-99). It is not within the scope of this review to describe these experiments, but it is indispensable to the discussion which follows to state one outstanding conclusion; that salt accumulation is dependent on metabolic activities of the plant cell associated with aerobic respiration, and reflected in CO_2 production. The amount of salt accumulated, however, bears no stoichiometric relation to the amount of CO_2 evolved by the accumulating cells. Emphasis is also given to the relation of the capacity of plant cells for constructive metabolism characteristic of cell growth and division, to sustained salt accumulation, especially the simultaneous accumulation of cations and anions not accounted for by exchange processes (review by Steward, 103).

The immediate interest of this review is in the accumulation of salts by roots. Young growing roots with available carbohydrate at their disposal, and suitably aerated, can accumulate certain ions, *e.g.*, potassium, bromine, chlorine, nitrate, with remarkable rapidity, provided that the root cells—the cortical cells are primarily involved—have not attained what may for convenience be designated as a “high-salt” condition; that is, a concentration of salt in the vacuole representing some sort of steady state, which retards or prevents further salt accumulation, except as new growth occurs, or as salt previously accumulated is translocated to the shoot (45).

The requirement of oxygen for salt accumulation is easily verified by simple experiments which may be done with excised root systems of barley or wheat, to avoid the many complications of root-shoot relationships. For example, experiments were carried out in which young barley root systems were immersed in a solution of potassium salt contained in closed bottles, or a stream of nitrogen gas was passed through the solution. Other comparable sets of roots were supplied with oxygen by passing a rapid stream of air through the solution. The unaerated roots accumulated little or no salt from the external solution while the aerated ones quickly built up high salt concentrations in the sap with parallel increases of con-

ductivity. Even within a few hours, highly significant salt accumulation can occur. It is important to note that both cations and anions can be accumulated in the sap by actively metabolizing root cells, and this results in an increase in chemical potential. The accumulation ratios, obtained by dividing the internal concentration of an ion by the external concentration, may become very large.

The oxygen requirements for salt accumulation vary greatly, no doubt, with the kind of plant (15), as well as with temperature, carbohydrate supply and other factors which affect the metabolism and the intake of salt by the root. In the experiments on excised barley roots, to which reference has been made, it was not essential for rapid salt accumulation to maintain nearly as high a percentage of oxygen in the gas passed through the culture solution as is present in the atmosphere. (In this respect, the barley roots differed from potato tuber tissue, but resembled potato roots (99).) More evidence is needed concerning the importance of CO_2 removal consequent on the aeration of the culture solution. Some preliminary experiments with barley roots gave the result that a concentration of CO_2 in the gas stream up to 10 per cent or perhaps higher did not retard salt accumulation.

The significance of oxygen supply in the culture medium for the absorption of salt extends, of course, to the soil, and Loehwing (56) has recently presented experimental data on soil aeration as favorably affecting plant growth and mineral absorption. It is his opinion, however, that injury may be induced by excessive aeration.

It is in accord with expectation, based on the intimate association between root metabolism and salt accumulation, that the temperature coefficient of salt accumulation by roots should be high ($Q_{10} = 2 - 5$) within ranges of temperature of chief biological interest, and under otherwise favorable conditions for rapid salt accumulation, such as initial low salt and high carbohydrate content in the roots and an adequate supply of oxygen for metabolism (45). High temperature coefficients for salt accumulation have also been reported for *Nitella* and potato tuber tissue (38, 93).

With the general concept of salt accumulation as a function of metabolizing root cells in mind, Prevot and Steward (77) attacked the problem of localizing along the axis of an individual root the regions most active in salt accumulation. Roots of barley, broad bean and cotton were employed. The absorbing surface of barley

roots was found to extend from the apex to the point of emergence of secondary roots. There was a longitudinal gradation of capacity for salt accumulation (bromide) with the segments near the apex attaining the highest salt concentration. It was concluded that the basis for this gradation "is to be sought in factors which determine the nutrition, growth and development of *cortical cells*" (77). Studies with methylene-blue indicated that the intensity of oxidation processes was greater in the apical segment than in older tissues. These researches on roots, and others already considered, suggest new approaches to some problems of physiological anatomy and of root pressure phenomena. Of great interest is the question of the relation of metabolic activities of roots to the removal of salt from the root cells in which accumulation first occurs, to the conducting tissues. Some type of active polarized salt movement seems to be required.

MECHANISMS OF SALT ACCUMULATION

Only a brief general comment can be made here on theories of mechanisms for salt accumulation, as they bear on the problem of accumulation by roots. Some of the most widely known theories of salt accumulation are based ultimately on hydrogen ion gradients between the internal (cell sap) and external (culture medium) solutions. The mechanism described in a previous review in this journal by Osterhout depends on activity gradients of [K] [OH] from external solution to sap, as far as the accumulation of potassium is concerned; other theories assume ionic exchange mechanisms by which hydrogen ions made available in the cell as a by-product of metabolism are exchanged for potassium ions of the culture medium (10, 11). The accumulation of anions receives less attention but is often explained as a mechanism of exchange, either of anions or of undissociated acids, with special emphasis on carbonic acid or bicarbonate ions. It has been found difficult to account for both cation and anion accumulation by these theories. The suggestion that the mere production of CO₂ by the cell, to maintain hydrogen ion gradients or to provide HCO₃ ions for exchange, serves in the mechanism of salt accumulation, is in disagreement with the evidence that anaerobically produced CO₂ is not effective in salt accumulation by roots or storage tissues even though it is produced in abundance for the purposes indicated.

Furthermore, *Nitella* or *Elodea* plants can accumulate anions (and cations) when the cells are illuminated and utilizing, rather than evolving CO₂ (38, 84).

Several of these theories have been applied with greatest emphasis to explain results derived from experiments or observations on *Valonia*. To what extent, then, can such theories serve as a general guide to a mechanism of salt accumulation by other plants? In answering this question, several points require attention. It is first to be observed that the mechanisms described by different investigators are not in agreement, even when they have certain assumptions in common. None of the mechanisms is accepted by Steward (103) who offers other interpretations of data cited in support of the various theories. (Compare discussion of *Valonia* experiments by Steward (102) and reply by Osterhout (70).) Steward and Martin (101, 104) conducted experiments on *Valonia* under carefully controlled conditions and also made and evaluated statistically numerous analyses of sap from *Valonia*, of two species, taken from the natural habitat in different locations. The general conclusion was that the evidence from these studies could not be reconciled with the gradient or ionic exchange theories of salt accumulation which had been proposed. Negative evidence has also been obtained by other investigators from experiments on *Nitella* cells (40, 49), *Chara* cells (21), barley roots and potato storage tissue (45, 103, and unpublished).

It is clear, in any event, that *Valonia* cells in the mature stages at which they have been studied have no capacity for increasing their vacuolar salt concentration to any degree comparable with that of actively metabolizing and accumulating root cells or other similarly active plant cells. Such cells have a high rate of respiration when aerated; the respiration rate of mature *Valonia* cells was reported as negligible (100, 101).

The preceding comments suggest some of the difficulties which are encountered when attempts are made to interpret all existing data on salt accumulation by living plant cells in terms of any simple mechanism. The complex reactions of metabolizing cells are concerned in solute accumulation, and further understanding of mechanisms will require the development of additional knowledge of the biochemistry of salt accumulation, of colloidal phenomena of the protoplasm, and of electro-potentials in respiring cells. (Lund

and his coworkers have stressed the importance of continuous bioelectric currents in roots for the production of which oxygen is necessary 57, 82, 83).

GENERAL ASPECTS OF RELATIONS OF HYDROGEN ION CONCENTRATION TO PLANT GROWTH

Not only the mechanism of salt absorption but many other phenomena of plant growth and of soil and plant interrelations have received interpretation in terms of hydrogen ion concentration. But as knowledge has expanded, the complexity of the system has become more and more apparent. Attempts to interpret the soil-plant system in terms of the single variable of hydrogen ion concentration of the aqueous medium are no longer so common as in the period following the general introduction of the concept of hydrogen ion concentration into plant physiological research. The profound importance of the consideration of soil reaction, of bases held in exchangeable form in soil colloids has become universally recognized. Much attention has also been given to aluminum toxicity in acid soils (54, 74).

Åslander (6), to mention only one recent paper by way of illustration, found that the fertility of soils of different pH values which he studied was correlated, not with the reaction of the soil, but with the ability of the soils to supply nutrients to plants.

OXYGEN SUPPLY TO ROOTS IN WATER CULTURE EXPERIMENTS

Returning now to the main theme of this review, the mineral nutrition of agricultural plants with special reference to controlled experimentation, let us consider further the relation of aeration of roots to plant growth and salt absorption. Plants of various species, and especially cereal plants, have long been grown successfully by the water culture technique without any provision of forced aeration of the nutrient solutions. Glass jars with tightly fitting corks, containing holes in which plants are securely held by cotton plugs, have commonly served as culture vessels. It might seem from the preceding discussion that under these conditions the supply of oxygen to the roots would be entirely inadequate. However, oxygen is introduced into the solutions when water is added to replace transpiration losses, and still another source of oxygen may, at times, assume importance, that of photosynthetically produced oxy-

gen translocated from shoot to root (16, 117). Finally, there are suggestions from work already mentioned that oxidation processes in the root may be facilitated by the nitrate usually employed as a source of nitrogen, or by catalytic metals.

Not all plants make satisfactory growth in stoppered jars, or even in loosely covered containers, without passage of a stream of air through the culture solutions, and even when shoot growth does not respond to forced aeration of the roots, its effects on root growth are nearly always pronounced. Development of very long fibrous roots is often observed, and the production of root hairs is favored. The anatomy of aerated and unaerated barley roots was given detailed study by Bryant (12), and characteristic differences between the two types of root systems were reported. General effects of aeration on the growth of tomato plants have been described recently by Arrington and Shive (5). Many species of fruit trees are particularly sensitive to deficiency of oxygen in the culture solution but may be grown successfully in water culture when continuous aeration is provided (19).

Some studies of the effects of aeration of culture solutions on plant growth were made by early workers (briefly reviewed by Arrington and Shive (5)), but many times the method of water culture has been utilized without definite assurance of the adequacy of the oxygen supply available to the roots, or of CO₂ removal from the culture solution. The importance of oxygen for the accumulation of salt suggests the possibility that conclusions concerning the relative physiological values of different nutrient solutions, derived from experiments with unaerated solutions, may not always hold for aerated solutions. Shive and his co-workers have developed during recent years a "drip-culture" method to insure renewal of supply of nutrient elements and to provide aeration (88). Shallow tanks or trays with a large surface for gas exchanges have also come into use. Sand cultures naturally offer better conditions of aeration than unaerated water cultures, which seems to explain, to a large degree, the different types of root growth in the two cases.

QUANTITATIVE RELATIONS IN NUTRIENT SOLUTIONS

At one period in the investigation of the mineral nutrition of crop plants, many workers sought to study systematically the effects on plant growth of varying by regular steps the composition or con-

centration of nutrient solutions. From experiments on a series of solutions it was hoped to discover a "best solution" or a small group of "best" solutions. These experiments stimulated great and valuable interest in the investigation of nutrient solutions, but it is now clear that in the interpretation of results several important factors were often omitted from consideration. Two of these have just been discussed: chemical elements effective in minute quantity and aeration of roots.

For some time there was also lacking a general appreciation of plant variability and the consequent necessity for showing that small differences in the growth of plants in different solutions were statistically significant. Davis (25) conducted experiments on wheat plants grown in several nutrient solutions of different composition, devised by other investigators, with many replications of the cultures. Yields were plotted in the form of frequency curves and probable errors estimated. Two of the solutions used, of markedly different composition, did not produce significantly different yields of plants. More recently, negative results have been reported from experiments designed to determine under soil conditions if some specific ratio of fertilizer salts was optimum (37). It may be added that even when nutrient solutions differ significantly, their physiological properties must be related to climatic conditions.

Another consideration which has led to re-evaluation of earlier data on ratios of salts in nutrient solutions is the rapid change in composition of nutrient solutions, or of soil solutions, which results from absorption of the solutes by growing plants. The total concentration and relative proportions of ions, because of selective absorption, are both altered. The initial composition of a solution, therefore, has no unique value. The total reserve of nutrients, which depends on the volume of accessible solution in relation to rates of solute absorption by the plant system under examination, may become more important than the initial composition of the solution, within a wide range of solutions. It is necessary to avoid a deficiency in the total available supply of nutrient elements, resulting in starvation effects. Flowing solutions, frequently renewed solutions, "drip" cultures and very large volumes of stirred solution for each plant, are now often employed to maintain a relatively constant composition in the nutrient solution, when it is the objec-

tive of the investigator to study concentration effects for different ions (for further discussion, see 52).

The foregoing discussion implies, not only that plants of a given kind can grow well in nutrient solutions of markedly different initial or maintained composition, but also that plants of diverse kinds can make good growth in nutrient solutions of the same composition, notwithstanding different requirements by the plants for particular mineral elements. Successful use has been made at the California Agricultural Experiment Station and elsewhere of one particular type of nutrient solution (44) for growing plants of agricultural interest, in great diversity. Another type of solution has been employed by Eaton (26), also for many kinds of plants. Certain of the nutrient solutions developed by Shive and his co-workers (87) are widely utilized in the study of numerous kinds of plants. Trelease and Trelease (112) have devised still other solutions in which combinations of nitrate and ammonium salts are present, and the hydrogen ion concentration stabilized through the selective absorption of ammonium and nitrate ions. These are but a few of many examples of different nutrient solutions which have been utilized by investigators. Others will be found described in texts on plant physiology.

While plants in general have great capacity to adapt themselves to widely different nutrient environments, it cannot be denied that unbalanced nutrient solutions, or solutions of too high total concentration, can easily be prepared, which, regardless of the adequacy of the supply of essential elements, are either toxic or incapable of producing the plant growth permitted by other factors of the environment. (The general question of ionic interrelations in absorption or of antagonism cannot be discussed in this review.) It is also true that special difficulties of maintaining iron in available form not infrequently arise in water culture experiments. High phosphate concentration may be unfavorable, particularly in early growth stages, not because of lack of balance of salts, but because of precipitation of iron in the solution, or possibly interference with its availability after absorption in the plant. The availability of manganese also is often of interest. Hydrogen ion concentration relations enter into the question of availability and the whole problem of iron and other chloroses, which has interested many investigators, is very complex and would require a separate review (53, 69, 81).

There is another aspect to the quantitative relations of mineral elements to plant growth. Plants may grow equally well in solutions of different composition, or of different supplying power for nutrient elements, and yet the inorganic composition of the plants may be profoundly influenced by the culture medium. A marked "luxury" absorption of some elements, for example, potassium, can occur. The question of the economy or efficiency with which nutrient elements are utilized in producing plant tissue has been raised many times in one form or another. Recently, Macy (60) has presented a concept of quantitative mineral nutrient requirements which embraces a "critical" percentage for each nutrient, varying with the kind of plant, a "luxury" consumption above the "critical" percentage, and a minimum percentage. The range between the point of "luxury" consumption and that of the minimum percentage is designated "poverty adjustment." Experimental data of various investigators are analyzed in the light of this concept and suggestions advanced with reference to the interpretation of laws of the minimum. Some years ago, Gericke (31) also discussed the question of minimum percentages of nutrients in plants. Thomas (106, 107) has recently considered the effect of one nutrient element on the absorption of another by the plant in relation to Liebig's law of the minimum. The literature of this general field of discussion is voluminous and often controversial. The present remarks are intended merely to draw attention to the problem of efficient utilization of nutrient elements. There also remain for discussion in other reviews the closely related topics of the relation of mineral nutrition to the "quality" of plant product and to reproductive processes, the absorption and utilization of mineral elements at different stages in the growth of plants, and effects of mineral elements on plant metabolism.

SALT ABSORPTION IN RELATION TO TRANSPiration AND TO ILLUMINATION OF THE SHOOT

In the early literature of plant physiology there was evident a general tendency to regard salt absorption as dependent upon transpiration. Later evidence did not support this view and the two processes have come to be regarded by many investigators as independent of each other (23). Recently, however, new data have been presented which are considered to be opposed to the conclusion that salt absorption does not depend on transpiration (85, 30).

Likewise with respect to the influence of illumination of the shoot on salt absorption by the root, opinions are divergent. Tottingham and his associates (110, 111) found that absorption of nitrate by wheat plants was accelerated by exposing the plants to light of short wave-length. Schmidt (85) made experiments under controlled conditions of humidity and was able to study the effects of shoot illumination on salt absorption at constant water absorption. The absorption of several of the ions of the nutrient medium was increased in rate as a result of illumination of the shoot, independently of transpiration. But Gile (33) reported, on the basis of a preliminary experiment, that nitrate could be absorbed as readily when plants were in the dark as when they were in the light. Nightingale and Schermerhorn (67) discovered that nitrate could be readily absorbed and utilized by asparagus plants in the dark.

These few citations give an idea of the perplexity which may well attend any survey of literature in an attempt to form a clear picture of the interrelations of light, transpiration and salt absorption. One difficulty is that the process of salt absorption by roots has not, in general, been envisaged as a metabolic function of roots, influenced by the shoot in several ways. The following considerations seem to be fundamental for the interpretation of data on salt absorption in relation to illumination of the shoot and transpiration, with particular reference to experiments of short duration on plants which have already reached a suitable stage of development under uniform conditions. When studies are carried on for long periods under differential environments, still greater complexity is introduced, since cumulative effects on growth become of importance.

(1) *Nutritional status of the plant.*

If the root cells have already attained a high concentration of salt as a result of conditions of previous nutrition, the capacity for further salt absorption is dependent upon either new growth of roots or on the translocation of salt from root to shoot. This translocation makes possible additional salt absorption, provided that the proper conditions for root metabolism are present. The prevailing view is that in the transpiring plant, salt moves upward with the transpiration stream (for discussion of this point and other views consult 23, 61, 18). Light and humidity could, therefore, influence salt absorption by roots through their control of transpiration de-

spite the fact that the actual process of salt accumulation by root cells does not depend in any direct way on transpiration. The experiments on excised roots already described make this point clear.

In any case, a proportionality between transpiration and salt absorption is not a necessity. It can readily be demonstrated that it is possible for plants to remove ions from a culture solution either more rapidly or less rapidly than water, depending upon the nature and concentration of the ion, initial salt status of the plant, metabolic activity of roots, and rate of transpiration. In one experiment with actively transpiring young barley plants, potassium was removed from the culture solution so much more rapidly than water that after 24 hours the concentration of this element was reduced almost to zero (47).

The growth and metabolic activities of the shoot in relation to its capacity for salt accumulation or utilization also demand consideration in any attempt to evaluate the influence of climatic factors in salt absorption. Mason and Maskell (61) suggest that nitrogen, phosphorus, potassium or other phloem mobile elements, if not retained in the shoot, can be re-exported to the root and retard the rate of salt absorption in relation to water absorption.

(2) *Available carbohydrate or other metabolites, or growth substances, essential to root metabolism and growth.*

Since the accumulation of salt by roots does not proceed in the absence of metabolism or capacity for growth, a supply of sugar, and possibly of other substances synthesized in the shoot, is needed for salt accumulation, and the indirect role of light in this process is therefore obvious.

(3) *Oxygen supply to the roots.*

The oxygen-supplying power of the external medium requires evaluation in terms of its adequacy for the particular plants under study. Merely bubbling air for a short time through culture solutions at infrequent intervals does not give assurance of adequate oxygen supply in the root environment. If the amount of oxygen available through the external solution becomes insufficient for root metabolism, internal aeration may play a role in some plants, according to evidence already cited that oxygen may be transported to the root from the illuminated shoot. Also inward movement of oxygen from the external medium could be accelerated by trans-

piration. The possible relations of nitrate or catalytic metals to oxidation processes have been mentioned.

(4) *Temperature of culture medium as affecting solute absorption and transpiration as affecting concentration of solutes in culture medium.*

In comparing the results from experiments in which the temperatures of the culture medium vary, evaluation of temperature effects on water and solute absorption should be made. Consideration may also be required of changes in the concentration of solutes resulting from effects of transpiration on the absorption of water, in relation to the absorption of solutes, although these changes may not be important when large volumes of culture solution are provided and the absorption period is very short. The question of the moisture content of the soil, as affecting soil solution concentrations, is presented in the next section.

Inasmuch as data on all these factors have not been available from the investigations so far published on effects of light and transpiration on salt absorption, it is not surprising that different conclusions have been reached from experiments conducted under different conditions.

The general point which it is sought to make can be further clarified by reference to some unpublished data. Sets of young barley plants, each constituting a large population and grown by a standardized technique, were divided into two groups for a salt absorption study, using continuously aerated nutrient solutions. One set of plants was placed in the dark and the other in the light, for a period of 7 hours. Within small limits of error, the amounts of potassium and nitrate absorbed were the same for each group. At the beginning of the experimental absorption period these plants had root systems low in salt and high in sugar as a result of the cultural treatment of the preliminary growth period. When oxygen was supplied to the roots, rapid absorption of salt began and continued until the roots had attained high salt concentration in the sap (primarily potassium salts). Movement of salt from root to shoot also occurred in the dark, in a humid atmosphere, under the influence of root pressure. Exudates may have a much higher concentration of mineral solutes than those of the culture solution.

The results of this experiment might have been given a general interpretation to the effect that absorption of salt is not influenced

by illumination of the shoot or by transpiration. But it is possible to do other similar experiments with plants in a different nutritional status, for example, high in salt and low in sugar, or experiments over longer time periods, and find that salt absorption is affected by illumination or by transpiration.

COMMENTS ON SOIL AND PLANT INTERRELATIONS WITH REFERENCE
TO ARTIFICIAL CULTURE EXPERIMENTS

Water and sand culture media can provide an entirely adequate root environment for the growth of most plants so far studied. If all the influential factors are properly adjusted, plants grown under water or sand culture conditions may even surpass plants grown in highly fertile soils, in both vegetative growth and fruitfulness. It follows, therefore, that no generally essential factor for growth is present in the soil and absent from the artificial media. Organic matter in the root medium in appreciable amounts, if at all, has not been shown to be an indispensable requirement for the growth of plants, however important secondary effects of organic matter in the soil may be. On the basis of general knowledge of the growth of crop plants in water culture, the idea has been suggested and extensive experiments made, to determine the feasibility of growing commercially certain kinds of crops in aqueous media, especially glasshouse crops (32). Sand cultures have likewise been utilized on a commercial scale by Biekart and Connors for growing floral plants (8). Eaton (26) has devised automatic methods of applying nutrient solutions to large sand cultures (compare also 116).

While the nutrition of the plant itself can be studied adequately without consideration of the soil system, it is another function of the science of plant nutrition to study the relation of soil conditions to plant growth. The question must be asked: what bearing do experiments with artificial culture media have on soil problems? In the soil, according to views generally held the immediate source of mineral nutrients for plant growth is the soil solution. Much effort, therefore, has been directed to the elucidation of the nature of this solution. The most direct approach is through the application of a displacement method (73, 13). The soil is packed in a tube at any desired moisture content and the soil solution displaced without dilution, by a column of water or alcohol, generally with the aid of air pressure. From the point of view of the soil chemist,

the solution so obtained is logically regarded as the soil solution characteristic of the mass of soil under examination, for the given moisture content.

The physiological interpretation of data on soil solutions, however, presents many difficulties. The determination of the composition of the solution, as it exists at one particular time, is generally not sufficient. The need is to estimate the "supplying" power of the soil for the various essential elements; in other words, the capacity to maintain concentrations of these elements above critically low values with reference to the particular requirements of the plant at each stage of growth. Furthermore, the soil solution prepared in the laboratory represents a composite solution from a heterogenous system which does not reflect accurately the physiologically effective solutions existing at points of root-soil contacts. The growing plant is, in a sense, an integrator of soil conditions, which no procedure of the chemical laboratory can imitate satisfactorily.

Plants are not passive in their relations to the soil. Throughout their growth, the roots disturb the physico-chemical equilibria of the solid and liquid phases of the soil by absorbing solutes (selectively) and by giving off carbonic acid formed in root metabolism. Jenny and Cowan (50) stress the displacement of bases held in colloidal complexes by hydrogen ions from carbonic or other biologically produced acids and suggest that these phenomena may lessen the importance of a simple soil solution theory for plant nutrition.

The moisture content of a soil during the growth of a crop is not constant and the relative composition and total concentration of the soil solution is subject to change as moisture is increased or decreased (13). A large body of evidence has now been made available in support of the conclusion that optimum moisture, so far as absorption of water by the plant is concerned, is not represented by any single value, but extends through a wide range of moisture contents between the wilting point and point of saturation (114). It is, however, possible that large changes in the concentration of solutes in the soil solution which result from changes in moisture content produced by plant transpiration may modify the nutrition of the plant. Nightingale and Farnham (68) were led to this conclusion by experiments on the blossoming of the sweet pea. (Compare also 28 & 80).

Notwithstanding the complexities of interpretation, soil solution studies have furnished indispensable knowledge regarding the soil-plant system. Important general relations of soil solutions to plant growth have been established, (14) and useful comparisons can be made between soil solutions and artificial nutrient solutions.

Soil solutions, even when derived from fertile soils, are generally very low in concentration of some essential ions, especially potassium and phosphate, among the ions required by plants in relatively large amounts. In nutrient solutions, the initial concentrations of these ions are usually much higher than in soil solutions, but this is a matter of convenience in providing a reserve of nutrients rather than an essential distinction between the two types of culture media. If phosphate or potassium are supplied in flowing solutions or in solutions of large volume frequently changed to prevent the reduction of concentrations to below critical values, very low concentrations of potassium or phosphate are adequate for plant growth, comparable with those found in many soil solutions. For some plants, a concentration of few parts per million of potassium and still lower concentrations of phosphate have proved effective in a number of investigations (52, 74, 92, 105, 113). Goedewaagen (34) was successful in growing wheat plants in solutions of very low nitrate concentration. Unpublished results of the present writer indicate that very low sulphate concentrations suffice even for plants of high sulphur requirements.

It is evident that plants can accumulate at rates necessary for good growth, some, at least, of the required nutrients, from solutions of great dilution, in artificial media or in the soil. But this accumulation depends upon many factors essential to the growth and metabolism of roots, as has been pointed out. Oxygen supply, suitable temperature, an adequate supply of available carbohydrate for root metabolism, toxic substances, all require consideration as well as the composition of the soil solution or the nature of the soil colloids. Also the ability of plants to withdraw solutes from the soil and their physiological requirements for these solutes cannot be adequately appraised without consideration of the influences of climatic and biological factors as affecting reproduction and root shoot interrelations. (For discussion of ecological factors consult monograph by Lundegårdh (58); also compare (47).

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THE BOTANICAL REVIEW

VOL. III

JULY, 1937

No. 7

CYTOTOLOGY IN ITS RELATION TO TAXONOMY

EDGAR ANDERSON

Missouri Botanical Garden, Washington University, St. Louis

Taxonomy and cytology study the same phenomena but from two very different angles. Cytology, or more properly karyology, concerns itself with the architecture of the germplasm; taxonomy with the adult forms which result from germplasms. The relation between the two disciplines can be illustrated by means of a simple analogy. The two viewpoints are like the insight into a family of strangers gained from (1) meeting the members of the family on the street or (2) looking in their cellar windows. The analogy is quite precise; the first is essentially the method of taxonomy, the second that of cytology. There is a big element of chance in the additional information gained by means of the new approach. In the language of the analogy one sometimes learns nothing new about a family from looking in the cellar windows; on the other hand, one sometimes finds evidence which puts the family in an entirely new light and clears up points which had previously been mysterious.

Continuing the analogy, it is sometimes difficult to see through the windows because they are small or dirty, as in the case of the Hamamelidaceae where cytological examination is difficult (Anderson and Sax, 1935). In other cases, the windows are large and clear, as in the genus *Tradescantia* where the chromosomes are enormous and the reduction divisions are easy to find. In every case the two views supplement each other; the taxonomic observations or the cytological data may be incomplete or partially in error, or one may be puzzled as to how the two sorts of information are to be reconciled, but there is no possible chance of real disagreement.

One of the simplest instances of the way in which cytological and taxonomic information supplement each other is provided by the American species of *Tradescantia* allied to *T. virginiana* (Ander-

son and Sax, 1936; Anderson and Woodson, 1935; Anderson, unpublished data). The basic sets of chromosomes in that group are practically indistinguishable from species to species by present cytological methods. Some of these *tradescantias* are diploids, that is, they have two sets of chromosomes; other species are tetraploid, that is, they possess four sets; still other species have diploid and tetraploid races. These races are morphologically indistinguishable* but when separated by cytological means it is found that the tetraploids are, on the whole, a little larger, considerably stouter, a little longer-flowering, a little hardier, and a little easier to grow under varying conditions. That these differences operate in nature and are of taxonomic consequence is shown when the distribution of the diploids is compared with that of the tetraploids (fig. 1). The diploid species are of limited distribution and even in those areas where they do occur are usually restricted to one particular

* And for that reason have been given no taxonomic designation.

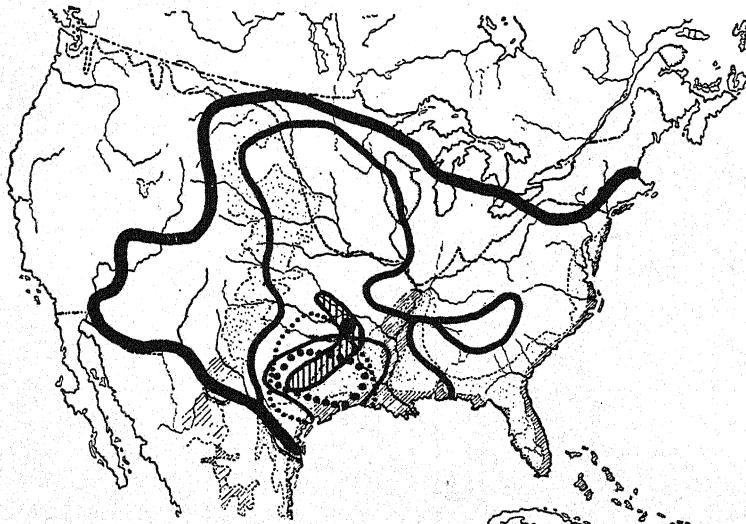


FIG. 1. Relationship between polyploidy and geographical distribution in the American species of *Tradescantia*. Outer heavy line: maximum distribution of tetraploid species; inner heavy line: maximum distribution of diploid species; heavy cross-lined area: minimum distribution of diploid species. Centered about this same area are the known diploid areas of species elsewhere tetraploid: large dots, *T. canaliculata*; small dots, *T. occidentalis*; unbroken line, *T. hirsutiflora*; solid black area, *T. ozarkana*. The stippling and cross-hatching on the map itself are indicative of relative geological age. Data from Anderson and Woodson, Anderson and Sax, and Anderson (unpublished).

habitat. By contrast, the tetraploid species and races have wide distributions and most of them have the ability to flourish under a variety of situations. By cytological methods, therefore, we can divide this group of closely related species into two categories, the diploid species and races, and the tetraploid species and races. Though they cannot be separated by orthodox morphological methods and, therefore, have been given no taxonomic designation, the diploids and tetraploids are so different physiologically that they fill somewhat different biological niches. The diploids are relatively invariable and most of them are extremely limited in distribution. The tetraploids are much more variable; they occur under a greater variety of ecological conditions and have much wider distributions.

These same cytological facts may also be used in determining the center of distribution of this group of species. For simple genetical reasons, diploids may quite readily give rise to vigorous tetraploids but tetraploids seldom give rise to diploids and if these do occur they are prone to be weak. In those species which have both diploid and tetraploid races we therefore know that the tetraploids must have originated from the diploids. *Tradescantia occidentalis*, to take a concrete example, is widely distributed throughout the Great Plains and eastern Rocky Mountains. It is a tetraploid throughout most of its range, though there is a small area in central and eastern Texas where a diploid race is found. On cytological and genetical grounds we know that the ancestors of these diploids must have given rise to the tetraploids, which in comparatively recent times have greatly extended the range of the species. It is, of course, conceivable that the diploids might since then have moved about considerably and the tetraploids did not actually spread out from this particular area. Such an argument is refuted by data from *T. ozarkana*, *T. canaliculata* and *T. hirsutiflora*, three other species which possess both tetraploid and diploid races. *Tradescantia canaliculata* forms the most effective comparison with *T. occidentalis* since the former reaches its southwestern limit in Texas while the latter is there at the southeastern extremity of its range. Nevertheless, the diploid races of these two species occupy almost the same identical territory, suggesting that it was from this actual area that *T. occidentalis* tetraploids moved out over the Great Plains and *T. canaliculata* tetraploids, to the Mississippi Valley. This conclusion is strengthened when one finds that the two other species, *T.*

hirsutiflora and *T. ozarkana*, exhibit the same tendency. Even more impressive is a comparison of this diploid area of species elsewhere tetraploid with the distribution of the species which are purely diploid (fig. 1). Using the method described by L. B. Smith (1934), the minimum area of the diploid species was determined. It is a narrow belt running from the Edwards Plateau in central Texas to the southwestern edge of the Ozark plateau in northwestern Arkansas. The diploid races of the four species mentioned above are centered upon this area, which from geological evidence is known to have been continuously habitable for land plants since very ancient times. Cytology, therefore, brings a new kind of evidence to join with that from geology and from geographical distribution. The combination of all three suggests very strongly that this area in the southwest was the immediate center from which the American tradescantias have developed in comparatively recent times. In this particular group, cytology, though of no assistance in discriminating between species, has given useful and unique information. It has shown that these species are made up of two physiologically different elements, the diploids and the tetraploids. It has, furthermore, given positive and independent evidence as to their center of distribution.

The case of these American tradescantias has been outlined in such detail because it is not an isolated instance but is certainly typical of very many genera of flowering plants. In a recent comprehensive survey of the phenomenon, Müntzing (1936) summarizes fifty-eight instances in which races differing in the number of sets of chromosomes have been discovered within the same species. In all cases, the morphological differences between these races was slight; sometimes, as in the case of *Tradescantia*, too slight to merit taxonomic recognition; occasionally it was worthy of varietal or even sub-specific rank. In all these genera, however, the same sort of difference existed as in *Tradescantia*. Under controlled experimental conditions the races with multiple sets of chromosomes (hereinafter referred to as polyploids) were somewhat larger, thicker, darker green, slower growing, hardier, and had less conservative distributions.

From the standpoint of floristics the most interesting case which has yet been reported is that of *Biscutella laevigata*, a European crucifer studied by Manton (1934). By cytological analysis carried

on in collaboration with the most recent monographer of the genus (Machatschki-Laurich, 1926), Manton demonstrated that this species is made up of two very different elements: (1) the diploids, a series of rare, more or less isolated, pre-glacial or inter-glacial relicts along the river valleys of central Europe, and (2) vigorous, aggressive tetraploids, occurring mostly on floristically youthful territory which was covered by the Alpine ice sheet during the Glacial Period. Similar though less detailed reports have been given for American Cactaceae by Stockwell (1935), for *Chrysanthemum* by Shimotomai (1933), for *Phleum* by Müntzing (1935), for *Empetrum* by Hagerup (1928) and for *Crepis* and related genera by Babcock (1936).

In the cases reported above, the polyploids were the result of duplicating the same basic chromosome set, and they are accordingly known as auto-polyploids. Much more dramatic changes are brought about by the duplication of more or less *unlike* sets of chromosomes, a phenomenon known as allo-polyploidy. Though it is known to occur in nature, the clearest demonstration is provided by a cultivated house plant. *Primula kewensis* originated in England as a sterile hybrid between an Abyssinian species of *Primula* and one from the Himalayas. It was attractive and winter-flowering and so, in spite of its sexual sterility, was propagated vegetatively. Eventually on several different occasions plants of this sterile hybrid gave rise to vigorous fertile branches whose seed, in spite of the plant's hybrid origin, bred true to type. Cytological study provided a simple explanation of the phenomenon. Each of the parent species had nine pairs of chromosomes. The sterile hybrid, as we would expect, had eighteen chromosomes, nine from each species, but they were too unlike to pair and form fertile gametes. The fertile branches, however, had just twice the number of chromosomes, thirty-six per cell; they had originated when a nuclear division had not been followed by a cell division and from the resulting giant cell there had grown out an allo-polyploid branch with two full sets of chromosomes, that is, two of each of the parental species. It could, therefore, form germ cells in a substantially normal fashion and was fertile and true-breeding. *Primula kewensis* is only one of a very large number of cultivated plants which are now known to have originated through allo-polyploidy (Schiemann, 1932).

The phenomenon is, however, not limited to cultivated plants. Müntzing (1936) has presented convincing morphological evidence to show that *Galeopsis Tetrahit*, a tetraploid European species, is an allo-polyplid derived from two other European species, both diploids. He has, furthermore, been able to prove his working hypothesis by actually synthesizing a duplicate of *Galeopsis Tetrahit* from the putative parental diploids.

Doubling of chromosome numbers in hybrids can take place quite as well when the parental stocks differ in their chromosome base number. To cover all such cases of fertile, true-breeding hybrids, resulting from doubling of the chromosome number, it is convenient to have a general term. While others have been suggested, the only one in general use is amphidiploid (Goodspeed, 1934).

One of the most interesting cases of amphidiploidy under natural conditions is that of *Spartina Townsendii*, a phenomenal inhabitant of north European estuaries and foreshores. Though it is known to have originated in modern times, it has within the last century not only spread widely but has produced a profound effect upon other coastal floras and faunae. Huskins (1931) has shown that its high chromosome number (126) is exactly what we would expect if *Spartina Townsendii* were an amphidiploid derived from the European *Spartina stricta* with fifty-six chromosomes and the American *Spartina alterniflora* with seventy. Since such an origin had previously been suggested from morphological evidence alone, the hypothesis, while, of course, still unproved, becomes increasingly probable.

Clausen (1933) and Keck (1932) have described an interesting case of amphidiploidy in *Pentstemon*, which will be even more significant when their combined taxonomic and cytogenetic study of that genus is published in full.

One of the most convincing cases of amphidiploidy is that of the American beardless irises, *Iris versicolor*, *Iris setosa* and *Iris virginica*. Anderson (1936) has shown that *Iris versicolor* has the chromosome number (108) which we would expect if it were an amphidiploid derivative of *Iris setosa* with thirty-eight chromosomes and *Iris virginica* with seventy. *Iris versicolor* is, furthermore, morphologically intermediate between the two putative parental species, though much closer to *Iris virginica* than to *Iris setosa*, as might have been predicted, since *Iris virginica* contributes nearly

twice as many chromosomes as *Iris setosa* and might, therefore, be expected to preponderate in the characters of the offspring. *Iris virginica*, furthermore, is centered upon a floristically ancient area in the southern United States and *Iris setosa*, the other supposed parent, has long been recognized as a typical glacial relict in the north (Fernald, 1925). The theory harmonizes so many otherwise incoherent facts from cytology, genetics, morphology, geographical distribution and glacial geology that it is at least well-established as a working hypothesis.

Amphidiploidy has a number of effects of taxonomic consequence. One of the most important is its effect upon the phylogenetic (and hence the morphological) relationships between species. An attempt is made to demonstrate this point diagrammatically in figure 2 which shows the course of phylogenetic development in a genus with and without polyploidy, on the simplest possible assumptions. The course of evolution is shown in the lower part of the figure. The species, as we see them at the present time, are the separate strands which run across the top of the figure. In

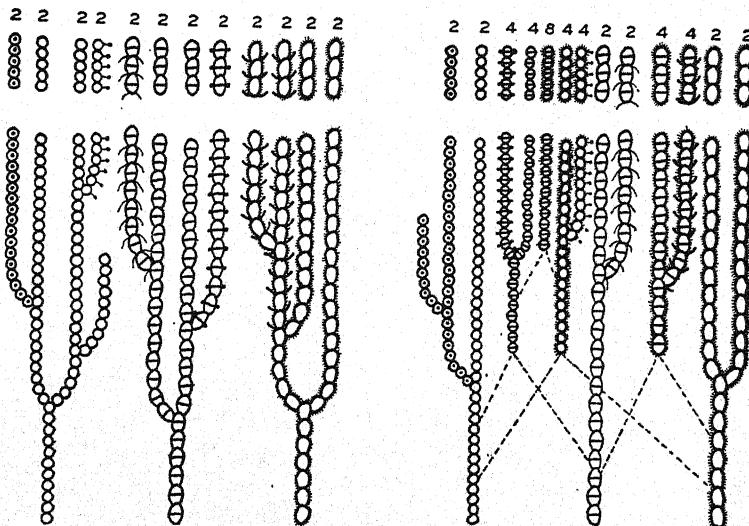


FIG. 2. Phylogenetic relationships with and without amphidiploidy. Branched figures at the base represent hypothetical development from three original stocks. Short figures above represent existing species and varieties. Numbers refer to number of basic sets of chromosomes (2 = diploid; 4 = tetraploid; 8 = octoploid). Further explanation in text.

the absence of polyploidy it is possible from morphological analysis alone to recognize that there are three separate stocks involved, the globular, the crossbar and the pubescent. By a careful inspection of differences within these three stocks it would even be possible to construct a hypothetical phylogenetic tree which would be very close to the actual history presented in the diagram below. With polyploidy, however, the distinction between the three original stocks is broken down. The morphological resemblances and differences between the species as we see them at present are reticulate and an attempt to reconstruct the course of phylogeny on a simple dendritic pattern would lead to gross error. For instance, were the difference between globular and oval species to be considered fundamental, one would have to hypothecate the parallel development, in both groups, of crossbarness and pubescence. If one, however, is provided with the chromosome numbers, as at the top of the diagram, it would be possible to reconstruct a substantially accurate phylogenetic diagram. The diploids would be recognized as fundamental and the tetraploids as derived from them, and the octoploid as originating from the tetraploids. In genera where there is no more amphidiploidy than in this diagram, it should be possible by a combined cytological and genetical attack to work out such phylogenies with a fair degree of accuracy. A number of such projects is already under way, and preliminary reports have been made on a number of interesting cases (Babcock, 1936; Goodspeed, 1934; Skovsted, 1934).

The genera and families of the flowering plants will, therefore, differ greatly in their fundamental phylogenetic patterns according to the prevalence of amphidiploidy. There are whole families, such as the Cyperaceae, in which it has not been found, in spite of intensive search (Heilborn, 1924, 1932; Hicks, 1928; Hakansson, 1928). There are many genera in which it is wholly absent, or in which it is at least exceedingly rare, as for instance *Rhododendron* (Sax, 1930) and *Quercus* (Sax, 1930). There are other genera in which it is about as common as in diagram 2, for example, *Iris* (Simonet, 1929, 1934). There are genera and families in which it is much more common. To this extreme group belong many of the Gramineae, the Solanaceae, the Labiate, and above all the Malvaceae. Polyploid relationships reach such a complexity in many genera of that latter family, as in *Sphaeralcea* (Webber,

1936), that it is no wonder that taxonomists have been in puzzled disagreement as to generic and specific limitations and relationships. The interested reader is referred to the indices of Tischler (1935-36) and Gaiser (1930) where reports of chromosome numbers for the angiosperms are summarized by families and genera. It is not possible from the mere report of chromosome number to know whether the polyploidy reported is auto-polyploidy or allo-polyploidy. Each, however, is an exceedingly common phenomenon. It is already safe to say that at least half the species and varieties of the flowering plants belong to polyploid series (Müntzing, 1936).

Chromosome number (or base number in the case of polyploid series) sometimes varies within the species, though such cases are rare. Occasionally, it is different in closely related species, as for instance in the genus *Scirpus* (Håkansson, 1928; Hicks, 1928). Much commoner is such a genus as *Crepis* or *Viola* in which the number varies within the genus but in which the related groups of species have the same chromosome number. Sometimes it is a character of even greater stability, as in the Pomoideae where only one base number (17) has been found in the entire sub-family (Sax, 1931). Chromosome number reaches its greatest stability in the gymnosperms (fig. 3) where the base numbers 10, 11, 12 and 13 are the only ones yet reported for the entire Coniferales, and the great majority of the genera belong either to a 12 series (Taxaceae and Abietaceae) or an 11 series (Taxodieae and Cupresseae) (Sax & Sax, 1933).

The taxonomic usefulness of information about chromosome number and the purposes to which this information can be put will, therefore, depend upon its stability in the particular group which is being studied. Babcock (1936) has found it useful in clarifying the relationships of *Crepis* with related genera. One of the most interesting cases reported to date is Whitaker's work (1933) on the Magnoliales and their allies. It will be remembered that here are included such families as the Magnoliaceae, Winteraceae, Schizandraceae, Trochodendraceae and Cercidiphyllaceae. They form a more or less natural group with certain obvious interrelationships. They are certainly very ancient and include such curious missing links as *Trochodendron* and *Tetracentron*. Whitaker was able to show that the genera he studied in these families fall into two series, a 19 series and a 14 series. These two groups, though some-

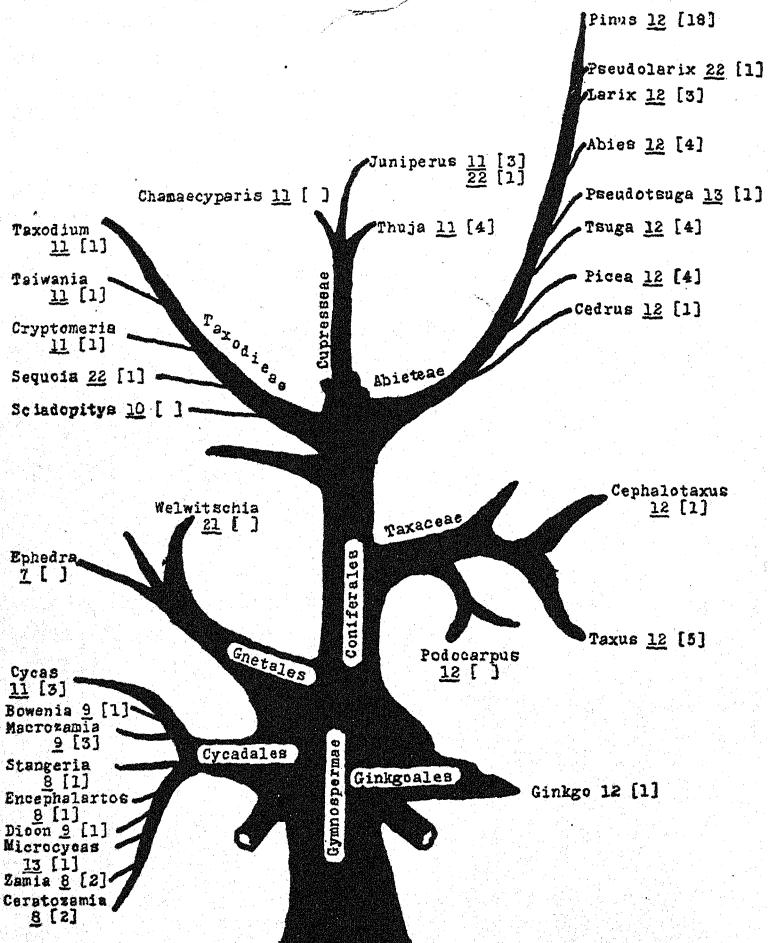


FIG. 3. Chromosome numbers and taxonomic relationships in the gymnosperms. Numbers in brackets refer to the number of species and varieties investigated. Data largely from Sax, and Sax and Beal.

what different from the arrangements of previous students (no two of whom have ever been in complete agreement), were, nevertheless, in accord with I. W. Bailey's unpublished evidence on nodal anatomy and with Wodehouse's (p. 331, 1935) pollen morphology. His evidence is particularly convincing, partly because the number 19 is so rare as a base number, and partly because Whitaker's grouping is in harmony with the known morphological facts.

Cytology sometimes gives decisive evidence on disputed taxonomic questions. Students of the Boraginaceae, for instance, have not been in agreement as to the proper disposition of *Brunnera macrophylla*, some holding that it well merited generic recognition (Johnston, 1924), others believing that it belonged in the genus *Anchusa*. Smith's (1932) cytological study shows very clearly that Johnston and Stevan were right in recognizing *Brunnera* as a distinct genus. Cytology in this case not only demonstrates that the chromosomes of *Brunnera* are distinct from those of *Anchusa*; the difference is of quite another order of magnitude than that found within the latter genus. Since S. G. Smith did not discuss

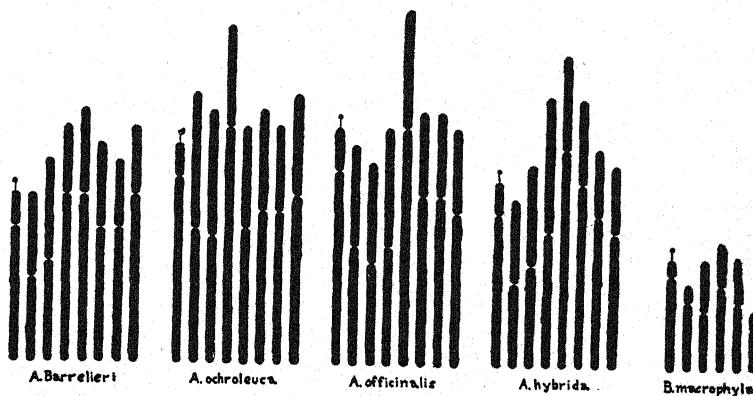


FIG. 4. Diagram of chromosome morphology in *Anchusa* and *Brunnera*, showing chromosome number, size, and position of attachment constrictions and satellites. Redrawn diagrammatically from Smith's published figures.

the evidence in detail and since Wright Smith (1933) has subsequently questioned the weight of the cytological evidence, the original figures have been redrawn diagrammatically but to scale and are shown in figure 4. One set of chromosomes is shown for each of four species of *Anchusa* and for *Brunnera macrophylla*. It will be seen that there are three kinds of differences between *Anchusa* and *Brunnera*: (1) size; (2) number, *Anchusa* having eight as a base number, *Brunnera* having six; (3) morphology, *Brunnera* having two chromosomes with sub-terminal attachment constrictions (in addition to the chromosome with a satellite) while there are no such chromosomes in *Anchusa*, which also has two to three with median or sub-median constrictions though there is only

one in *Brunnera*. A triple difference of this magnitude within a genus would be extremely rare among the angiosperms. For the Boraginaceae, which are relatively conservative in such matters, a cytologist would confidently predict that *Anchusa* and *Brunnera* could not even be neighboring genera.

A similar instance is provided by McKelvey and Sax's (1933) discovery that *Yucca* and *Agave* with a few related genera have identical chromosome complements, an arrangement of five very large and twenty-five very small chromosomes, so distinctive that it is unthinkable that it could have arisen independently in both the Liliaceae and the Amaryllidaceae. Cytology, therefore, offers strong evidence in favor of Hutchinson's (1934) treatment of these genera as a distinct family, the Agavaceae. The cytological evidence might be carried even further to indicate relationships between the Agavaceae and other families of the monocots. Whitaker (1934) has already presented a preliminary discussion and further facts (though without comment) have been presented in such summaries as that of Matsuura and Suto (1935). Hutchinson's stimulating work has shown what radical changes may be necessary if we are to have a natural classification of the monocots. The time is ripe for some qualified investigator to undertake a comprehensive study of their generic and family relationships, utilizing the facts from morphology, geographical distribution and cytology.

It is difficult to set limits to the eventual usefulness of cytology in discriminating between species and genera. With advances in technique it has been possible to add such gross features of chromosome morphology as the satellites and the position of the attachment constrictions to the bare facts of chromosome number. In certain insects it is now possible, by means of the salivary gland technique, to examine the fine structure of the chromosomes. Bauer (1936) has shown how relationships in a whole family, the Chironomidae, may be studied by this technique and he has already made a beginning at assembling the necessary data. Dobzhansky (1937) has shown that not only can closely related species be effectively diagnosed by this technique (Dobzhansky & Tan, 1936), but that morphologically indistinguishable races within the same species may be readily identified. He has even been able to plot the geographical distribution of races within the species and, what is even more important, to demonstrate beyond a reasonable doubt the developmen-

tal sequence of these intra-specific races. With the rapid advances which are taking place in plant cytology it should not be long before similar studies will be possible in plant material.

Even with the relatively crude data already at hand there is a very large number of genera of the flowering plants where cytological data would be a useful guide to taxonomic relationships if carefully determined and used in conjunction with other evidence. Wright Smith (1933, p. 181) has discussed the cytological evidence obtained by Brunn (1932) for the genus *Primula* in the light of his own taxonomic studies in that genus. He concludes that in monographic studies "the last word lies with the morphology. But I can record without hesitation my obligations to the cytologist. His contribution has been of the greatest interest and value. But only by marshalling *all* the data, morphological and cytological, can a final judgment be made."

With Wright Smith's general position (see also Smith, 1936) that cytology merely contributes another bit to the sum total of morphological facts, the reviewer is in complete agreement, *in so far as purely morphological contributions of cytology are concerned*. Cytology is unique, however, in that it may indicate not only a difference between species or groups of species but may also demonstrate the way in which these differences came about. It is evidence as to the architecture of the very germplasm itself and is, therefore, of more fundamental importance than the mere architecture erected by that germplasm. Cytological evidence, therefore, can do more than discriminate between species; it can illuminate species.

Any monographer who has had intensive experience with more than one or two families of plants knows that there are characteristic peculiarities of relationship in different families and genera. The sharpness of genera, the morphological relation of one species to another, the degree to which genera can be divided and subdivided in clear-cut natural groups, are matters in which there are wide differences between certain of the families of the flowering plants, as for instance between the Cruciferae, the Liliaceae, the Malvaceae and the Orchidaceae. In other words, the very pattern of evolution differs from group to group. Cytology is of importance to taxonomy because it is already able to diagnose certain causes of these differences in the pattern of evolution. It is already able to discuss authoritatively such matters as reticulate phyloge-

nies, which had previously been perceived by only the most acute taxonomists and about which there is not yet any general agreement among taxonomists. With the rapid advances in cytological technique which are taking place at the present time, it will not be long before cytology is able to play an even more important rôle in separating special factors from general factors. The cyto-taxonomist of the future, after a few routine examinations, will be able to estimate for a systematist, in advance of the morphological information, the probable evolutionary pattern in the genus or family he is about to monograph. He will, furthermore, supply the key to such puzzling problems as the Pomoideae, the Malvaceae, the genus *Agropyron*, or the genus *Festuca*. These are typical examples of many groups of flowering plants which in the opinion of the reviewer cannot be adequately monographed without *correlated* cytological and taxonomical research. Such pioneer work as Erlanson's on *Rosa* (1933) and Ruttle's on *Mentha* (1931) has demonstrated the absolute need of cytological information in the taxonomic interpretation of critical genera.

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PIGMENTATION IN PLANTS, EXCLUSIVE OF THE ALGAE*

M. MÖBIUS

Frankfort a/M, Germany

The means by which Nature produces her great variety of colors in the plant kingdom are fairly simple in principle. They involve only a few pigments, and other contributing factors are the structure of the colored organs and the distribution of the pigments within the tissues. The nature of the colors is substantially different, moreover, from that of colors displayed in the animal kingdom, for, whereas the gorgeous hues of beetles, butterflies and birds are brought about for the most part through surface structures and interference phenomena, in the plant kingdom it is much more a matter of pigments and but rarely of superficial metallic luster. The pigments of plants may appear in an unadulterated state, for the most part in the form of crystals, or they may be dissolved in the cell sap, or combined with protoplasmic carriers or with the cell walls. They can also be secreted from the walls or can originate through transformation of the latter. In discussing the various colors we shall note examples of these types and shall also see how different a color may be, even with the same pigment and produced in the same manner, for example, in red cell sap, according as it alone is contained in the organ or tissue or is combined with other coloring materials, for instance, with chlorophyll, or according to whether it exists only in the epidermis, or the inner layers, or in both. Other factors, too, may be involved and they all may account for various shades, whether in solution or in solid form. In addition, air enclosed in the intercellular spaces also has a profound bearing by diminishing transparency, and the thickness of the cell walls is of influence upon the intensity of the colors contained within the cells.

If different pigments occur together it is possible for a median tone to appear and Exner has differentiated addition- and subtraction-colors between the tones so produced. The former originate when two colors are simultaneously perceived by the eye and thus

* Translation from the original German by the editor and approved by the author.

produce a combined effect; for instance, when red cell sap and yellow chromatophores in the same cells of *Tropaeolum majus* make the petals appear orange-red. Subtraction colors, on the contrary, appear when two differently colored layers lie one over the other, and when, in such cases, one part of the incident white light is absorbed by the upper layer, another part by the lower layer, and only the residue of the light is reflected. This is the situation in the black or brown colors of some blossoms. Smooth surfaces can produce a glossy shade, whereas epidermal cells, which have developed into papillae, cause an intensification of the color. Thus we see how very important is the histological structure of an organ with regard to the color it presents to the eye.

So far as the nature of the individual pigments and of their carriers is concerned, we can consult textbooks and handbooks. In this paper we are concerned more with the methods employed by plants in producing their color impressions. We shall see, moreover, how on the one hand the same coloring material, anthocyanin for example, can produce in all gradations the most contrasting of colors, red and blue to violet and black, and, on the other hand, how the same color, especially black, can be produced by the most varied of methods.

GREEN

We shall therefore discuss the individual colors in a definite sequence, beginning with green as the characteristic and most widely distributed in the plant kingdom. In spite of the endless variety of shades which are to be seen in the wild and which play so important a part in the art of gardening, green is universally and with very few exceptions produced in the same manner, namely, through the well-known chlorophyll in the chloroplasts. This chlorophyll, according to Willstätter's investigations on more than 200 species of plants, is always of the same chemical constitution. To be sure, the proportion of the two constituents composing chlorophyll, the green and the yellow-green components, is not always the same. The ratio of the green to the yellow can vary, according to Willstätter, between 6:1 and .3:1. A leaf will, therefore, appear either darker or possess more yellow according to the proportion of these pigments contained in it. Histological relations play, however, a much greater rôle. Cells with transparent walls and otherwise

uncolored contents allow the chlorophyll to shine through without modification, as in an alcoholic solution. Masses of *Spirogyra* filaments or submerged leaves of *Nuphar luteum* exemplify this unmodified appearance of chlorophyll. In leaves of land plants there is, in most cases, a colorless epidermis over the chlorophyll tissue and larger or smaller intercellular spaces in the latter which, as has already been mentioned, naturally influence the effect of the coloring material. Other factors modifying the green appearance are the number of layers in the palisade tissue and in the spongy parenchyma, the amount of chloroplasts in the cells and their distribution along the cell walls. The last-mentioned factor is demonstrated by the well-known experiment of fastening a strip of tinfoil to a leaf exposed to the sun. Various parts of a leaf by their differences in structure and in the amount of chlorophyll may bring about what is known as a piebald effect. Furthermore, a soft and wrinkled leaf displays entirely different tones of color than does a smooth leathery leaf. It need hardly be mentioned how pubescence and the secretion of wax, of calcium salts and of other substances change a pure green into gray-green, for such considerations belong rather to the chapter dealing with the gray colors. Likewise, we shall later consider how the autumn coloring of foliage into yellow, brown and red comes about. At this point we may yet call attention only to the winter alteration of color in so-called evergreen plants. This change, according to Kraus, is brought about by a variety of causes, involving the appearance of anthocyanin, the distribution and aggregating of the chloroplasts and an alteration in the chlorophyll pigment itself in that it changes from a blue-green to a brown-green tone.

As in the case of broad leaves, normal chlorophyll is altogether responsible for the color of green stems, of phylloclades, of green aerial roots and of all vegetative organs engaged in photosynthesis, as well as of sepals or petals, pistils, fruits and seeds when they appear green. In green peas, for example, it is the cotyledons of the embryo which are green and visible through the seed coat, the latter containing very little chlorophyll.

The above-mentioned exception where green is not occasioned by chlorophyll, is exhibited by certain fungi, including several Hymenomycetes with green pileus or green stalk. Not much is known regarding the chemical nature of this green pigment or of

its cytological position. The yellow-green color of the cap and stalk of *Leotia lubrica*, according to Zopf (1893), is produced by the combined effect of a copper-green crystallizing pigment, a yellow-brownish probably resinous body, and a yellow lipochrome.

Most remarkable of all is the green moldy wood caused by the fungus *Chlorosplenium aeruginosum* which contains in the walls of its hyphae an intensive copper-green pigment known as xylochloric acid. We would finally mention the bacterioviridin of the so-called Chlorobacteria, which is distributed in the plasma, particularly in its periphery.

We need yet draw attention only to the fact that in plants green is never produced through a mixture of blue and yellow, as can be done in the art of painting; at least, I know of no case where blue anthocyanin and yellow anthoxanthin produce a combined effect of green.

YELLOW

Yellow, however, is quite different for it may be produced through the medium of different pigments, either associated with the chromatophores or the cell wall, or dissolved in the cell sap. The former are known as anthoxanthin bodies and are responsible for the color in the majority of yellow flowers, occurring for the most part in the epidermis but also in the mesophyll. In yellow vegetative organs, on the other hand, the anthoxanthin bodies are more abundant in the inner tissues.

The yellow leaves of etiolated plants contain, instead of green chloroplasts, smaller yellow-colored plastids whose pigment is supposed to be identical with protochlorophyll which is regarded as an early stage of chlorophyll and which can be converted into the latter through oxidation.

It is quite a different matter with regard to yellow autumn coloring of foliage for in this case one finds in the mesophyll cells filled with watery cell sap not degenerated chloroplasts but yellow strongly refracting drops which have absorbed the yellow pigment of the disintegrated plastids. This is exemplified, for example, in *Ginkgo biloba* whose leaves appear pure yellow in autumn, and in *Fagus sylvatica* whose leaves after losing their color shine in the sun like old gold. The varied coloring in yellowed leaves of autumn is brought about by variations in the contents of carotin and xantho-

phyll, and the proportions of these components, as in yellow garden varieties and in the so-called formae *aureo-variegatae*, are so varied that we cannot describe them in detail.

The dissolved yellow pigments are contained in fat or oil, or in the watery cell sap. Fatty pigments are to be found especially in fungi, for example, in *Polystigma pulvrum*, *Peziza bicolor*, *P. aurantiaca* and in *Spathularia flava*. Pollen cells are often colored with yellow oil which apparently accumulates in them after being produced by disintegration of the tapetal cells of the anther. Though there are many examples of this, pollen cells are not always colored through the medium of such oil, but, in many cases, by their yellow exine.

The yellow pigment dissolved in the cell sap is known as anthochlore and is found in flowers and fruits. It cannot definitely be determined merely by outward inspection whether flowers are colored yellow by anthoxanthin or by anthochlore, though the latter is more generally found in those of a bright yellow color. Two species of *Primula* supply us with very good examples of the foregoing, the bright yellow *P. elatior* containing anthochlore, while the much deeper yellow *P. officinalis* carries anthoxanthin bodies in its petals. This at the same time exemplifies the fact that systematic relationship has nothing to do with the nature of pigments. There is also an instance, noted by Geitler (1934), where the yellow color of blossoms is produced by yellow cell walls. Otherwise, however, yellow pigments in the walls are more generally found in broad-leaved foliage, for instance, in leaves having a yellow edge, such as those of *Erythronium japonica*, species of *Agave* and in *Phormium tenax*. But many pure green appearing leaves, as those of *Taxus baccata*, *Nerium Oleander* and *Cycas revoluta*, possess such yellow outer walls, which, according to Stahl's conception, serve to weaken to a great extent the passage of blue and violet light. It is not easy to prove, however, that this conception is correct.

Pigmentation of the cell walls is responsible also for the yellow color in certain woods, such as fustic, *Cotinus Coccinea*. The fisetin contained in this wood is a heterocyclic compound, but other membrane pigments belong to other chemical groups, so that the name xylochrome for wood pigments is unjustified from a chemical viewpoint. Yellow-colored walls are found also in fruit skins, such as that of *Prosopis strombulifera*, in seed coats, such as in

Sinapis alba, in sporangia and spores, in pollen grains and finally in fungi, e.g., *Hygrophorus conicus* with a yellow-colored cap. In lichens, for example, *Evernia vulpina*, the yellow pigment of the membrane is stored up and this is likewise true of the so-called gold and silver colors. On the underside of the leaves of *Gymnogramme argentea* var. *aurea* are excretory hairs which produce on their swollen ends crystalline deposits of a yellow shade. This excreted substance, according to chemical investigation, is not a true wax.

ORANGE

Similar to yellow is orange which can be produced either by means of simple pigments of this color or through a combined effect of different pigments. The beautiful orange-colored sepals of *Strelitzia reginae* offer a fine example of the former since the epidermis as well as the mesophyll cells are similarly filled with very fine rod-shaped chromatoplasts of an orange-yellow color. Such chromatoplasts, though not always of the same form and distribution, are to be found also in the petals of *Erysimum Petkofianum*, in the blossoms of *Cucurbita* and of *Narcissus poeticus*, in the fruits of *Lycopersicum esculentum*, in the arillus of the seeds of *Evonymus latifolius*, and, as is generally known, in the roots of *Daucus carota*. Lipochromes of an orange-yellow color are found in fungi and lichens, but only once have I observed an orange-yellow pigment dissolved in the cell sap, namely, in epidermal cells of the petals of *Papaver nudicaule*.

With regard to other ways and means of producing the orange color, namely, by combination, the best example is the orange itself, that is, the fruit peel of *Citrus aurantium*. In this case there is a combined effect produced by the large excretory cells containing a bright yellow oil, the cortical and epidermal cells provided with yellow-red chromatophores, other epidermal cells containing red anthocyanin, and finally the yellow cuticle. Red cell sap and yellow chromatophores can occur in the same cells to produce an orange color, or they may occur in different cell layers superimposed upon one another. Examples of this are to be found in the petals of *Cheiranthus Cheiri* and of *Tropaeolum majus*, as well as in the fruit pulp of *Hedychium Horsfieldii*. The orchid *Ada aurantiaca* is peculiar in having both yellow cell sap and red chromatophores in the same cells of the perianth segments. Anthochlore, too, can pro-

duce the yellow ingredient of orange, as in *Carthamus tinctorius* where it is to be found in the epidermis of the blossoms, while the inner cells contain anthocyanin, thus presenting a good example of the origin of subtraction colors.

BROWN

Next to green, brown is the most common color in the plant kingdom, generally the color of older dead tissue. On the death of parts of the plants as well as in the brown autumnal coloring of leaves, the plasma with its chloroplasts as well as the walls become colored. A similar browning comes about in rot and fungal injury. The brown substances are designated phlobaphenes and are supposed to be manufactured first in the cell sap through oxidation of resinous materials and are then thought to be absorbed by the plasma and the cell wall. They derive their name from the fact that they occur chiefly in the cortex, accounting for the latter's brown appearance. Without entering any further into their chemical nature we shall mention a few examples wherein only portions of the individual cells are brown when the whole organ appears to be so. In most cases, brown membranes are involved, as in the coating of many fruits and seeds, and in the spores of ferns, mosses, lichens and fungi. In the case of fungi and lichens it frequently happens that also the vegetative parts, the hyphae or the entire thallus, are brown because of the cell wall, and in the mosses this may be true of capsule walls, of the setae and of the rhizoids. As cases where organs are colored brown through the medium of the cell wall we may mention the leaf stalks of *Adiantum reniforme*, the roots of *Helleborus niger*, the leaf sheaths of *Restio tectorum*, the stipules of *Ficus pandurata* and the appendages on anthers of *Viola tricolor*. The walls of hair cells are also responsible, as a rule, for a brown pubescence on otherwise green parts, as in many ferns and flowering plants.

Brown chromatophores are rare, but do occur in *Orobanche* and in certain orchids, such as *Neottia*. Brown cell sap, known as anthophaein, was first known in the case of the brown flecks on petals of *Vicia faba*. It occurs also in the petals or sepals of *Delphinium* species and is characteristic as a blossom pigment in the Coelogyne tribe of orchids. Similar brown pigments, not identical with anthophaein, however, occur in such a variety of plants that we cannot

instance them here. The combined effect of chlorophyll and red anthocyanin can also account for a brown color in leaves or perianth segments and the distribution of the two pigments among the cells and tissues is very variable, as we have already learned in the case of the orange. We shall not cite any examples of these but will only mention in conclusion that a brown coloring can be caused also by incrustation with iron oxide and that this occurs in algae, lichens and bacteria.

RED

Red is very common in all groups of plants and in all organs, and can be produced by any of the means available to plants for the production of color. In most cases, the anthocyanin dissolved in the cell sap produces a red color but this pigment is not a single homogeneous substance, as was formerly believed. On the contrary, there is, according to Willstätter's investigations, a great number of anthocyanins which can be grouped into several categories. Thus, a certain shade of red characteristic of a particular plant depends upon the chemical constitution of the anthocyanin. There may also be involved, as in other cases, differences in concentration of the pigment and in its occurrence in different layers of tissue. As a rule, red cell sap is found in the epidermis, as in petals, in leaves of blood-colored varieties and in red stems. In other cases, the epidermis is free of anthocyanin, the latter occurring then, as in radishes (*Raphanus sativa radicula*), in the outermost and innermost cortical layers of the root. In the red beet (*Beta*) all the parenchyma cells appear to be filled with red cell sap.

Anthocyanin, dissolved in the cell sap, also produces the red autumn coloring of certain leaves, but its part varies according to the species to the extent that the epidermis may be colorless and only the palisade cells contain the red sap. Or, both tissues may contain the pigment, and the spongy parenchyma, as well, may take part in producing a color effect.

Anthocyanin can also be held in the cell wall, and red-colored walls, the chemical nature of whose pigment is not well known, are common. Red chromatophores are not very prevalent in higher plants. We have already mentioned those of the orchid *Ada aurantiaca* as well as those of the Florideae, but these will be further discussed elsewhere. Red chromatophores have been found in a variety of leaves and are generally known to be present in the red hypanthium of the roses, the so-called hips.

BLUE

It is well known that anthocyanin is colored blue through the influence of alkalies and that it is the same pigment which at one time appears red, at another time, blue. We can observe this in Nature in the same species, as in *Anagallis arvensis* or in *Salvia pratensis*, in which there are red and blue flowering forms, and still better in many of the Boraginaceae where the blossoms are red in their early stages and later appear blue. Pure blue is not very often met with in the plant kingdom, at any rate less frequently than red.

As is true of red anthocyanin, blue also appears in the epidermis of flower petals, e.g., in *Linum usitatissimum*, *Centaurea cyanus*, *Lobelia erinus* and in *Gentiana acaulis*. From these examples one can perceive how varied the different tones of the same pigment may be apart from minor differences in chemical composition. The pigment can also occur in solid form, as in the blossoms of *Amsonia angustifolia*. Small blue grains appear to color the petals of *Strelitzia reginae*, but these may be small blue vacuoles containing blue sap. In blue corn the anthocyanin is closely associated with the aleuron grains.

Other blue pigments are rare, and we would mention only the well-known bluing of certain fungi, especially at the junction of the cap, in species of *Boletus*. According to recent investigations of G. Bertrand (1933), this is caused by the oxidation of a phenol-like substance, boletol. Blue cell walls, too, occur in fungi and algae. In higher plants, for instance, in the so-called blue fir, a coating of wax can cause the leaves to take on a blue tone.

VIOLET

Violet is similar to blue and may be produced directly by the anthocyanin assuming a tone intermediate between red and blue; in some cases, however, it originates through combination. As an additive color it appears in *Convolvulus tricolor*, in the violet markings of its petals whose epidermal cells sometimes carry red cell sap, at other times blue or even violet sap. I have observed violet as a subtractive color in *Viola odorata* where the epidermis on the underside of the lower petal contains blue anthocyanin, while the subepidermal layer contains red anthocyanin. The subepidermal layer on the upper side likewise contains red anthocyan, but the epidermis itself is colorless. There are other instances in which the

violet color is produced by the cell wall, as in the exine of pollen grains of *Goethea Mackoyana*, the water roots of *Pontederia crassipes*, the lower leaf surface of certain species of *Riccia* and particularly in the ventral scales of *Marchantia polymorpha*. Among fungi there belong here the sclerotium of *Claviceps purpurea*, known as ergot, and probably some mushrooms.

BLACK

Of particular interest, it appears to me, is the origin of a black color, for it is brought about in so many different ways that we cannot mention them all but must refer to my work of 1920. We therefore give here only in a very general way the following causes that may account for the color black: colored plasma, anthocyanin, anthophaein, combination of several pigments already mentioned, pigments in the cells and of a nature not very well known, colored cell walls, and substances lying outside the cells. The last-named are the phytomelanes which, according to the investigations of Hanauseck, arise from the middle lamella and represent a form of carbon and are to be found in fruits, in involucral bracts and paleae, and in the roots of certain Composites.

GRAY

Gray might perhaps be designated as a distinct color but it plays no very great role in the plant kingdom. Integral gray pigments are not known, gray color being derived either by a combination of bright-blue or bright-violet sap with golden-yellow grains, as in some floral leaves, or through air-containing tissue lying over the cells which have brown or green contents. Gray foliage, peculiar to many plants and especially noticeable in the olive, is produced through hairs with aeriferous cells on the otherwise green blade, or by virtue of many intercellular passages in the mesophyll. This condition obtains also in the case of many lichens, for instance, in *Peltigera canina* which appears gray when in a dry state, but green upon being submersed when the air is replaced by water. Very remarkable is the development of a silvery-gray color in old wood used for building purposes and long exposed to the air. My investigations have shown that a fungus with brown hyphae becomes established in rather deep layers in the fiber cells and that with the aid of overlying aeriferous cells it produces the gray color. Ac-

cording to the same principle, the silver-gray in the gills of the inky-cap mushroom, *Coprinus atramentarius*, is produced: the spores supply the brown background while the cystids and the air spaces between them are responsible for the somber layer covering it.

WHITE

Enclosed air also plays the essential role in the production of white. This we find in porcelain-like white petals, for example, of the camelia, consisting of a colorless aerated tissue which becomes quite transparent, however, when the air is replaced by alcohol. In general, those parts of plants whose walls and cell contents are colorless appear more or less pure white. This is true, for instance, of wood with air-containing cells and as well of any tissue possessing colorless contents and colorless walls, as in many endosperms. In edelweiss and certain other plants the white appearance is attributable to a covering of hair and this role can be taken over also by wax, sodium chloride or lime.

GLOSSINESS

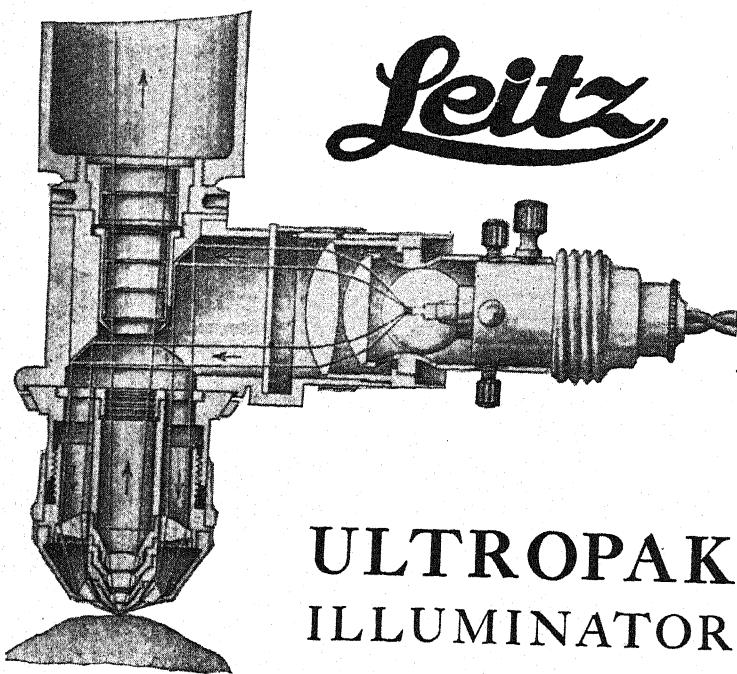
We might thus conclude an explanation of the origin of colors, but it is fitting to say a few words concerning the origin of various types of gloss, characteristic of many parts of plants. By means of the air enclosed in a tissue there may be produced not only a whitish color but also a silvery sheen, especially in leaves where the stratum of air lies between the colorless epidermis and the assimilation tissue. There are numerous examples of this and also numerous modifications in the position of the air stratum, according as it lies more to the outside or more within the leaf. We shall not discuss those cases wherein other factors may result in a silvery or metallic sheen. The velvety sheen so noticeable on many floral leaves is effected by the papillose form of the epidermal cells, whereby, as has already been mentioned, the color located in the epidermis becomes more intense. In leaves with colorless epidermis the papillose form, responsible also for a velvety sheen, is supposed to be an adaptation, according to Stahl's conception, for rapid shedding of rain water from the upper side of the leaves. If the epidermal cells develop into true hairs they can bestow a silky or silvery luster, as in *Convolvulus cneorum* and *Elaeagnus parvifolius*. As a result of the vesicular hairs on *Mesembryanthemum crystallinum*

linum there arises a so-called drop- or water-sheen, while a perfectly smooth epidermis bestows a glassy sheen, as in the rubber tree, *Ficus elastica*. Fifty years ago I described how the oily sheen on yellow-flowering species of *Ranunculus* is produced by the oil-bearing epidermis and by the underlying starchy layer. In certain leaves of tropical plants there is a lacquered sheen which, too, is actually brought about by a resinous substance given off by the leaf. The blue sheen displayed by some leaves and fruits, for instance, the leaves of *Selaginella laevigata* and the fruits of *Viburnum tinus*, is caused by the combined effect of structures and of distribution of the pigment, but it is so varied and complicated in individual cases that a description of all the cases would carry us too far afield. We wish to draw attention only to the occurrence of this feature as well as to the different types of sheen and thereby show how multifarious are the colorings in the plant kingdom caused by such phenomena.

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THE BOTANICAL REVIEW

VOL. III

AUGUST, 1937

No. 8

SYMBIOTIC NITROGEN-FIXATION BY THE LEGUMINOSAE

P. W. WILSON¹

*Department of Agricultural Bacteriology and Agricultural Chemistry,
University of Wisconsin*

INTRODUCTION

Research concerned with symbiotic nitrogen-fixation is conveniently divided into three periods in which the type of problem that has attracted major emphasis has varied. The pioneer labors of Liebig, Lawes and Gilbert, Boussingault, Ville, Atwater, and of others initiated the first period which was concerned primarily with the specific question: Are green plants able to utilize free nitrogen? This period came to an abrupt close in 1886 with publication of the classical paper of Hellriegel and Wilfarth in which it was demonstrated that leguminous plants in association with certain bacteria were capable of fixing atmospheric nitrogen, but that non-legumes, in general, did not possess this property. Intense effort directed toward practical application to agriculture of the implications of this discovery characterized the second period. For forty years, agronomic phases of the problem were intensely explored with almost complete neglect of matters of more purely "theoretical" interest. During this period, immediate questions of husbandry were answered largely through empirical studies, and inquiries directed toward an understanding of the more fundamental aspects of the nitrogen-fixing process were few in number and the results of doubtful value.

For the past decade it has been increasingly apparent that the answer to many problems of applied agriculture await solution of at least a few of the basic questions which heretofore have been disregarded—empirical research directed toward specific and often

¹ John Simon Guggenheim Memorial Foundation Fellow, 1936. The author expresses his appreciation to the Foundation for its grant to conduct research into the problem of nitrogen-fixation by bacteria in consultation with European authorities.

local field problems may have reached the point of diminishing returns. Studies on the biochemistry and mechanism of biological nitrogen-fixation may eventually prove economical, even though the immediate results are of chief interest to the so-called pure scientist. At present, several laboratories and experiment stations are engaged in definite programs specifically designed to investigate the mechanism of the process, and others have included in their research program, problems which supply collateral information. Definitely, a third period has begun in which theoretical aspects of the problem are receiving greater attention, even though concern with the practical questions of the second period still retains the major emphasis.

The purpose of this review is to present a critical summary of the progress made in the third period, especially as it is concerned with fundamental problems of biological interest rather than practical considerations. Research of the first and second period has been discussed in the monograph of Fred, Baldwin and McCoy (37). In order to avoid an excessive bibliography, this monograph will be used as a reference whenever it is necessary to mention the older work as a background for the discussion.² Also, since the mechanism of nitrogen-fixation by the free-living organism, *Azotobacter*, has been recently reviewed by Burk (23), reference to non-symbiotic aspects of biological nitrogen-fixation will be restricted to those which have a definite relation to similar studies on the symbiotic problem. Other recent reviews concerned with the general problem of biological nitrogen-fixation are given in references: (72, 46, 93).

PHYSIOLOGY OF THE ORGANISMS

Nitrogen-Fixation

It is customary in writing reviews of current research to include "recent advances" in the title, but it is doubtful if such a claim could be substantiated for many phases of the root nodule work unless by advances are meant the abandonment of positions which seemingly were well established by past studies. As an example of this type of advance may be cited the research dealing with the question: Are the root nodule bacteria (*Rhizobium* sp.) able to fix atmospheric nitrogen in the absence of the host plant, i.e., when

² Such references will be indicated by the year of the publication rather than by a number.

grown on laboratory media? Early investigators took an affirmative answer more or less for granted and apparently had little difficulty in obtaining positive results. Occasionally, the evidence was negative, but little attention was paid to these discrepancies. The validity of the belief that rhizobia are able to fix nitrogen nonsymbiotically was challenged in 1929 with the publication of three independent researches (Hopkins, 1929; Allison, 1929; Löhnis, 1930) in which all attempts to secure fixation by the organisms when cultivated on a variety of laboratory media were unsuccessful. An analysis of 50 papers made by Wilson, Hopkins, and Fred (125) revealed that although in 29 of these, claims of fixation by the bacteria alone were made, the majority of such claims is open to serious question because of possible errors in the technique. Such errors include: (a) question of purity of culture used; (b) insignificant fixation of nitrogen—less than one milligram per 100 cc. of medium; (c) insufficient replication of samples; (d) use of inadequate methods of analysis. For example, it was demonstrated by repetition of the experiment that the widely cited positive results of Golding (1905) might have arisen from inadequate sampling of the heterogeneous substrate (plant tissue) used. Likewise, Olaru's (1915) positive results with bean extract medium containing manganese salts were subject to error through loss of nitrogen from the controls during incubation—a loss not sustained by the inoculated cultures because of growth of the bacteria.

Since 1932 the few papers (30, 51) which have appeared that are concerned with fixation of nitrogen by rhizobia alone have presented uniformly negative results. Winogradsky (127) inoculated silica gel plates each containing 100 mgm. of glucose plus salts with organisms from nodules of pea, vetch, alfalfa and clover; the total nitrogen after incubation was determined by Pregl's micro-method. Under these conditions, about 10 per cent of the sugar was used and gains of nitrogen of .003 to .053 mgm. per plate were observed. Addition of NH_4Cl , asparagine, or peptone to the medium brought about increased utilization of the glucose but losses of total nitrogen up to .30 mgm. per plate; however, with an extract of yeast or peas, gains as high as .132 mgm. per plate were observed. Because of the small increases noted when the organism was grown alone, together with the erratic nature of

the gains and losses when combined nitrogen was furnished, these data hardly indicate fixation. Nevertheless, the detection of *small* but *consistent* increases of nitrogen in inoculated media by use of the refined methods of micro-analysis may offer possibilities for proof that the organism is *capable* of fixing nitrogen when growing alone.

If it is not possible to demonstrate fixation by the bacteria apart from the host plant, the use of excised nodules is next suggested. Wilson *et al.* (125) report negative results with constantly aerated pea nodules supported on glass beads. Use of a gasometric method for analysis rather than the traditional Kjeldahl procedure was made by Galestin (38) to detect uptake of free nitrogen by lupine nodules contained in a closed system. In five experiments no significant fixation was observed. Recently there was brought to the writer's attention a hitherto neglected paper on this subject by Krascheninnikoff (52) which appeared in a collection of Russian scientific works published in 1916. Krascheninnikoff used a gasometric method, the accuracy of which was determined as $\pm .15$ cc. of nitrogen; through use of large quantities of nodules (15 to 20 grams) and high pressures of oxygen this Russian investigator observed losses of nitrogen (.095 to 2.42 cc.) from the atmosphere in 16 of 21 experiments. It is probable that the high rate of respiration induced by the increased pO_2 , together with the large quantity of tissue and the delicate method of analysis employed, enabled this worker to observe uptake of elemental nitrogen which either did not occur or escaped detection in the experiments by later investigators.

Because of the inability to demonstrate fixation of nitrogen by the bacteria alone, alternative mechanisms for the symbiotic fixation have been proposed; among the favored ones is that the plant itself is the responsible agent. This suggestion is merely a special case of the time-honored controversy of the last century as to whether or not green plants in general can use the nitrogen of the air. In spite of periodic set-backs the idea is a recurrent visitor to the literature of plant science—its latest appearance is of especial interest because of its bearing on symbiotic nitrogen-fixation problems. Vita and collaborators (95, 96, 97) reported that during germination, seeds of the lupine, pea and horsebean fix appreciable quantities of free nitrogen if exposed to certain stimulants which in-

clude CO, alkaloids and even dilute solutions of iron, manganese, potassium and magnesium sulfates.

In experiments made with Alaska peas, Orcutt, Shannon and Wilson (65) could not confirm the observation; through replication, these workers were able to make a statistical analysis of their data which showed that all observed gains were within the variations of nitrogen content of the peas used. Confirmation of Vita's work was reported by Haritantis (42), but Girtschanoff (40), in an elaborate series of tests, reported negative results and once more stressed the importance of replication with subsequent statistical evaluation of the data. Meanwhile, Wilson and co-workers (77) extended their studies with samples of the Italian varieties of peas with which Miss Vita had secured the positive results. They confirmed the claim of Vita that during germination an increase in the nitrogen content of the peas as revealed by the Kjeldahl method may occur, but demonstrated that their own positive results arose from two sources: (a) variations in the nitrogen content of the seed; (b) errors inherent in the Kjeldahl method of analysis. It was shown that various Kjeldahl methods do not always estimate quantitatively certain forms of organic nitrogen known to be present in biological materials. For example, the official Kjeldahl method gave low results with dry pea seeds as compared with the Dumas method. However, if water or H₂O₂ were added to the seeds prior to digestion, a higher value for the nitrogen content as estimated by the Kjeldahl procedure was obtained, and the apparent gains observed on germination were so reduced as to fall within experimental error. More recently, Skallau (76) reported negative results, but his technique differed materially from that used by Vita. His assumption that her positive findings arose from errors in calculation was shown to be false by Wilson (118).

These findings emphasize the inadequacies of the Kjeldahl method for nitrogen-fixation studies in which the evidence necessarily must consist of *differences* in nitrogen content—at the start and end of the experiment—of a substrate high in complex organic nitrogen compounds. If the nitrogen compounds are subject to change during the course of the experiment, the observed fixation may arise solely from differences in the quantity of nitrogen estimated by the method. De Rossi (73), concerned with the allied

problem of non-symbiotic nitrogen-fixation by *Azotobacter* and other soil microorganisms, has arrived at identical conclusions regarding the applicability of the Kjeldahl method to experiments in which the criterion of fixation is the *difference* in nitrogen content of substrates high in combined forms of this element. The need of statistical analysis of the data from such experiments is stressed in the experiments of Reuszer (71). When examined in the light of these criticisms, the recent claim by Dhar (34) of non-biological fixation in Indian soils as well as many of the older experiments with rhizobia growing alone lose much of their significance.

Growth and Respiration

Among other results originating from the negative findings with respect to fixation by the bacteria in the absence of the host plant, has been an increased interest in the physiology of the organism, especially in the growth and respiratory functions. If the organisms are the responsible agents in the nodule, and if failure to demonstrate nitrogen-fixing activity outside the host rests merely on ignorance of the necessary environmental conditions, then a more intimate knowledge of the metabolic activities of the bacteria appears essential for ascertaining the postulated required environment. Previous physiological studies on the organisms have been characterized by the use of qualitative or semi-quantitative procedures, *e.g.*, their ability to grow on or to alter the pH of a given substrate. Recent studies emphasize the quantitative aspects of the activities of the cells.

Georgi and Wilson (39) studied the growth and respiration of organisms isolated from the nodules of pea, clover, alfalfa and soybean, and found that the rate of respiration, glucose used, and glucose appearing as CO₂, increased with the pO₂ of the atmosphere. However, excellent growth and respiration were noted with pressures of oxygen of less than .05 atm. This latter observation has received confirmation through work of Zycha (128) and Rabanova (67). Virtanen, Nordland and Hollo (110) report that *Rh. trifoli**ii*, the organism from clover, will ferment glucose in the absence of oxygen if masses of "resting cells" are used, and that products of the anaerobic carbohydrate breakdown include lactic, butyric and acetic acids, ethyl alcohol, H₂ and CO₂. Similar results were obtained through use of nodules from peas grown under

bacteriologically controlled conditions. The normal aerobic carbohydrate metabolism of these bacteria produces primarily CO₂, H₂O, and gum.

Walker, Thorne and collaborators have made an extensive survey of the growth and respiratory functions of several species of the root nodule bacteria, employing the Warburg apparatus in short-time experiments. They have given especial attention to the influence of carbon (60, 61), source of nitrogen (113, 114), and of the reaction of the medium (81) on rates of growth. Although the importance of such studies should not be minimized, many of the data do not admit of easy interpretation since the growth functions are difficult to separate from those of respiration. The resting cell technique offers a solution for this difficulty since with non-proliferating cells respiration alone is under consideration (49). Preliminary investigations by Wilson (120) indicate that this technique offers considerable advantages over other methods for characterization of the oxidative mechanism of the organisms.

Physiological studies of more general interest on the root nodule organisms have been concerned with the influence of accessory growth substances on initiation of growth by the cells when placed in certain media. Soon after the isolation of these organisms in pure culture it was learned that their development on laboratory media was greatly stimulated by extracts of soil or plants. Various extracts have been proposed including those of yeast (37), asparagus (29), kraut juice (4), mold tissue (110), potato (74) and leguminous plants (47)—each possessing particular virtues according to the claims of the proponent. Whether the source of these virtues depended on nitrogen constituents or some accompanying unknown substances has not been determined. This aspect of the nutrition of the bacteria was attacked by Allyn and Baldwin (1930) who inclined to the view that a function of the extracts was to present a suitable environment with respect to the oxidation potential of the medium. They showed that growth could be initiated in an unfavorable medium, *e.g.*, mannitol-nitrate broth, if reducing compounds as cysteine, thioglycollic acid, ferrous salts, and even non-specific substances as sand, agar, and filter paper were added. The action of all of these was interpreted as effects on the oxidizing character of the medium (12).

A preliminary paper by Allison, Hoover and Burk (9) an-

nounced the discovery of a factor in commercial cane sugar which when added to a mineral salt, c.p. sucrose— KNO_3 medium, greatly stimulated the development of root nodule bacteria. They stated that the factor is essential for growth and respiration of these organisms and because of its direct effect on respiration, together with certain other properties, proposed that it be designated as *Co-enzyme R*. Later publications dealt with its almost universal occurrence in plant, animal and soil extracts, its effect on growth (10), its preparation in a partially purified state from *Azotobacter* cultures, and some of its properties (43). These latter include: its stability toward heat, acids and alkalis; its solubility in absolute alcohol but not in ether, chloroform or benzene. Purified preparations were found to be effective in quantities as small as .4 parts per million. Since *synthetic* humic acids were ineffective in stimulating growth, irrespective of the iron content, the stimulating effect of *natural* humic acid on the growth of rhizobia when added to the sucrose-nitrate medium was ascribed to accompanying quantities of co-enzyme R rather than to the iron content (11). It was suggested that the results obtained by Allyn and Baldwin through addition of filter paper, agar and sand may have been caused by the accessory substance carried as an impurity (10).

Confirmation of the existence of an accessory substance which stimulates growth of the root nodule bacteria was soon made by Thorne and Walker (80), but these authors questioned whether the data imply the existence of a co-enzyme essential for respiration. This preliminary study was followed by detailed investigations on the growth and respiration of the root nodule organisms in various types of synthetic media (82, 83, 84). Their major conclusions from these researches are:

1. The c.p. sucrose- KNO_3 medium is particularly unfavorable for the development of rhizobia because of its low iron content and the use of KNO_3 as a nitrogen source.
2. If available iron is added to the medium, and if asparagine or ammonium salts are substituted for the nitrate, continuous culture of the organisms is possible without addition of accessory substances.
3. There is no evidence that any accessory growth factor is *essential* for growth or respiration of rhizobia, but certain substances do *stimulate* growth of these organisms in media composed of highly purified materials.

4. Since the inability of rhizobia to initiate growth in the nitrate medium can be overcome by addition of reducing compounds, and since these as well as the accessory growth substances reduce the initial respiratory quotient of the organisms, it is suggested that one of the important functions of such substances is to provide an initial hydrogen donator to the cells.

5. This added hydrogen donator lowers the oxidation-reduction potential of the medium and provides an initial readily available source of energy.

6. Because the beneficial effects appear to reside in a number of agents rather than in one specific substance, and because there is no evidence of the essential nature of any of these, use of the term *Co-enzyme R* is inadvisable, and the more general designation of *accessory growth substances* is proposed.

Further work on this problem has immediate interest not only for researches on the root nodule organisms but also for those in the allied fields concerned with growth hormones, stimulants, as well as for various other problems of nutrition of plants and micro-organisms. Especially are answers to the following questions of primary importance:

1. Is the factor (or factors) essential or only stimulatory?

The data of the two groups of workers discussed in the preceding paragraphs are not necessarily in conflict, since the factor may be only a stimulant in favorable media but essential for initiation of growth in an unsuitable environment.

2. Is the effect primarily on general growth or on respiration? Since the published data relevant to this query have been entirely concerned with growth measurements, little can be said with respect to the claim that the effect is specific for respiration. Existence of an earlier response in the micro-respirometer than is observed in cell increase is not conclusive evidence that effect on respiration has preceded growth, unless the latter is restricted to cell division. Use of non-proliferating cells in order to differentiate between growth and respiratory responses, together with comparison of rates, instead of total activity, might prove advantageous in these studies.

3. What are the iron requirements of the organisms?

4. Is there one specific, or a variety of compounds, that can function as the accessory factor? It should be noted that the

description of all the properties of the substances used by various workers do not agree (43, 48).

5. What is the mechanism of the action of the factor? The work of Thorne and Walker has proved suggestive in this respect and deserves full consideration. Nevertheless, it appears improbable that the extremely small quantity of purified material known to be effective can materially alter the oxidation-reduction properties of a medium so highly poised as is the nitrate broth. Also, it is suggested that the function of the substance may be that of a hydrogen carrier in the sense used by Green, Stickland, and Tarr (41) rather than a hydrogen donator.

PLANT-BACTERIAL RELATIONSHIPS

Cross-inoculation Groups

The question of whether a single or several species of bacteria are concerned in the symbiosis with leguminous plants has been a subject for controversy since the isolation of the organisms by Beijerinck in 1888. Early workers, having no evidence to the contrary, assumed that a single species was involved, and the first experiments, performed in a manner that practically ignored the factor of contamination, apparently confirmed this belief. As the technique of soil bacteriology advanced, errors in the former studies became obvious and the idea of one species of organism acting as a universal invader was supplanted by the opinion that each genus of plant requires a specific organism. It was soon apparent that this point of view was too narrow and the idea of cross-inoculation groups was developed. The basic concept of cross-inoculation grouping is that the Leguminosae can be divided into small groups of genera (usually closely related), each of which requires a particular species of organism for its symbiont. For a time this solution appeared to be satisfactory; then came disquieting reports that certain exceptions to the universal application of the cross-inoculation hypothesis had been observed. Unfortunately, these exceptions to the cross-inoculation concept could not always be repeated, even by the original investigator, so they were readily dismissed by that final refuge for the inexplicable in bacteriology—contamination.

The chief source of difficulty has been the cowpea and soybean groups, the former consisting of a large collection of many genera

which are only very distantly related taxonomically. Whereas the other groups generally show little tendency to violate cross-inoculation rules, the behavior of the twenty-one genera listed by Fred, Baldwin and McCoy (37) as comprising the cowpea group is so erratic that these authors judged it inadvisable to designate a specific bacterial species for this group, as was done for six of the others, *viz.*, pea, clover, alfalfa, lupine, soybean and bean. That the soybean organism, *Rhizobium japonicum*, could form nodules on the cowpea, *Vigna sinensis*, was demonstrated by Leonard (1923), but efforts to bring about the reciprocal cross, *i.e.*, infect soybeans with organisms isolated from the cowpea, were unsuccessful. Since Leonard's experiments were made in a bacteriologically controlled environment, dismissal of the data as arising from contamination was hardly a logical deduction, and reinvestigation of the cross-inoculation problem was undertaken at several experiment stations.

Recent studies have been characterized by isolation of new strains of organisms from native wild leguminous plants in different areas. In this way it has been possible to eliminate the complications which may arise from continuous association of certain species of bacteria with one genus of the leguminous plant brought about through either artificial or natural inoculation of cultivated crops.

Outstanding contributors to this problem together with the particular areas used for source of native wild leguminous species are: Carroll (29), Florida; Conklin (31), Massachusetts, Connecticut and New York; Raju (68), India; Allen and Allen (7), Territory of Hawaii; Bushnell, Sarles, and Fred (28), and Reid (70), Wisconsin. The great majority of the wild leguminous species examined in detail to date have been placed tentatively in the cowpea group since organisms isolated from the wild plants formed nodules on *Vigna sinensis*; the reciprocal cross frequently was not attempted and when it was, not always successfully. Since the results of all these investigations are in essential agreement, the individual studies will not be reviewed but only a summary of the findings given. Under certain conditions reciprocal crossings between the soybean and members of the cowpea group are observed and much less frequently crossings involving members of the lupine and dalea groups and those of the cowpea group are noted. Cross-inoculation is not clear-cut; both *strain variation* among the bacteria and *host specificity* in the plants are observed. For example, certain strains of the

soybean organism are able to invade only one or two members of the cowpea group, whereas others will infect nearly all members that have been tested. Likewise, certain plants appear to be immune to organisms except those isolated from the same genus or species; others are readily invaded by organisms isolated from different genera. *Vigna sinensis* appears to be the "universal recipient" in the group, *i.e.*, the cowpea is the most susceptible species to invasion by bacteria isolated from other species or genera. The existence of a "universal donor"—an organism which will consistently infect all the genera included in the cowpea group—appears doubtful. Within the cowpea group as now constituted reciprocal cross-inoculation between members exclusive of *Vigna sinensis* is extremely irregular.

However widespread is the acceptance of the experimental facts, their interpretation shows no such unanimity of opinion. Carroll (29) concludes, ". . . present day conceptions of, and practices in, legume inoculation are illogical and unsound and should be revised." Walker and Brown (115) propose that members of the soybean and cowpea plant groups be combined, and that *Rh. japonicum* be adopted as the species name for the organism. Conklin (31) rejects such a step at this time unless the present bases for determining species and groups be changed. Allen and Allen (7) also emphasize that the general conception of a cross-inoculation group is one in which the bacterial species are mutually interchangeable and until evidence is presented which demonstrates that reciprocal crossing is of general and not exceptional occurrence, consolidation is inadvisable. They also remark on the inadequacy of the data dealing with "effectiveness" (ability to fix nitrogen) in the cross-inoculation studies made to date and are of the definite opinion that irrespective of its importance for classification purposes, the factor of effectiveness is decidedly pertinent to agriculture, and therefore deserves consideration. Reid (70) also stresses the practical implications of combining the soybean and cowpea groups. He contends that the relationship between the soybean organism, *Rh. japonicum*, and the organism isolated from *Vigna sinensis* is closer than are many of the relationships among the bacteria isolated from other plant members of the cowpea group; hence, the two groups are not distinct and the bacteria probably represent only physiological adaptations rather than separate species. Nevertheless, he believes

separation of the two groups should be maintained as evidence that the particular adaptation found associated with a given host plant is the proper one for use in agriculture. Raju (68) also disagrees with the suggestion of Walker and Brown since he believes that if the soy bean is admitted to the cowpea group, inclusion of members of the dalea and lupine groups would be the next logical step—a logic which may lead to even more than the present confusion. Inability to secure reciprocal cross-inoculation is sufficient in his opinion to keep the groups separate.

An important contribution to this problem of cross-inoculation grouping has recently been made by Reid (70) and by Raju (69) who have demonstrated that the environment of the plant, especially length of day, intensity of light, and presence of combined nitrogen, may determine whether a strain of the organism can invade a given host plant. The erratic and often contradictory responses noted by previous investigators may have resulted from neglecting to control these factors.

In summary, it appears that the present status should be maintained until such time as the various cross-inoculation studies within the cowpea group can be made from the point of view of effectiveness as well as infectiveness. The observation that all species in this unwieldy collection do not exhibit reciprocal cross-inoculation may not prove to be unique for this group only when more extensive researches are applied to the other, especially with organisms isolated from wild species of the host plants (28, 31). The facts that certain strains of the bacteria from all groups lose the ability to infect when cultivated under certain conditions on artificial media (56, 124), together with the unexplained lack of nodules on the sub-family Caesalpinoidea of the Leguminosae (5, 6), indicate that much more basic information is necessary on the broad front of how and why certain leguminous plants are invaded by these particular organisms before anything resembling a permanent scientific classification of the rhizobia will be possible.

Host Plant Specificity

Implicit in the original concept of the cross-inoculation groups was the idea that all members of a given group were invaded and enabled to fix elemental nitrogen with equal ease by all strains of the proper species of bacteria. Later studies showed that this quan-

titative notion of the equality of the strains must be replaced by a more qualitative one since marked differences in the ability of strains to benefit the host plant were found. Although studies of strain variation have been carried out primarily from the point of view of variability in the bacteria, evidence has accrued that the rôle of the host plant must also be considered in accounting for the diverse relationships exhibited between invader and host (16, 37, 104).

Recent studies (20) specifically designed to investigate the function of the plant indicate that marked variation in the quantity of nitrogen fixed may occur with a single strain of the proper organism when used to inoculate not only different genera of plants associated in the same cross-inoculation group but also different species or varieties of the same genus. The extent of this type of variation is still unknown, but it appears that it may be restricted to the interaction of certain strains of the organisms with different host plants. For example, with *Melilotus* several strains of *Rh. meliloti* were equally effective when used with five species and two varieties, but two strains of bacteria showed marked variability in quantity of nitrogen fixed with different hosts. Similar results have been obtained with five species and five varieties of *Medicago*; the variable strains noted with *Melilotus* were likewise involved in the erratic behavior of the *Medicago* species.

Bacteriophage

Another complicating factor in the host-bacteria relationship has been revealed by discovery of a bacteriophage specific for the root nodule organisms. The phage appears to be widely distributed, and races isolated from nodules or members of the same or of different cross-inoculation groups may lyse a given culture of rhizobia (53). The first investigations on the bacteriophage for rhizobia were confined to laboratory studies of lysis, occasionally accompanied by speculations regarding its possible rôle in symbiotic nitrogen-fixation. It was suggested that strain variation of the bacteria might be correlated with the sensitivity of the strain to lysis, but experiments by Laird (1932) and by Wilson, Hopkins and Fred (124) failed to verify this suggestion. Extension of these investigations by Almon and Wilson (14) once again demonstrated that cultures of *Rh. trifolii* classed as "good" with reference to their ability to

benefit the host plant included both phage-sensitive and phage-resistant strains; the same was true of cultures classed as "poor." These experiments were made in the absence of added bacteriophage, but addition of this agent with the organism used to inoculate the plant altered the relation between host and bacteria. For example, in the presence of phage, fixation of nitrogen was unaffected only when: (a) the bacterial strain was resistant to phage; (b) the bacteria were sensitive to phage but "poor" with respect to nitrogen-fixation. If the organism used, however, was both sensitive to phage and a "good" strain, fixation of nitrogen was decreased and only resistant bacteria were recovered from the nodules formed.

Similar results are reported by Demolon and Dunez (32, 33) who ascribe "fatigue" of alfalfa soils in France to the presence of a bacteriophage for the root nodule organisms. As a result of laboratory and field experiments they concluded that in an alfalfa "fatigued" soil the root nodule organisms had disappeared because of lysis by the phage. They suggest that as long as the bacteriophage exists, alfalfa will fail on the soil. After a time, however, the phage should disappear as it is sensitive to desiccation, insolation, anaerobiosis, and temperatures below 10° C. When this occurs, normal development of the alfalfa should be obtained by inoculation of the seeds at time of planting with an efficient strain of the proper bacteria.

Although the observations of Vandecavaye and Katznelson (94) on the presence of the specific bacteriophage in the soil and alfalfa plants from "alfalfa sick" fields in Washington differ from those of the French workers only in details, they consider the conclusions of the latter to be too sweeping. Because of the occurrence of secondary growth of resistant strains of the organism, it appears unlikely that complete bacteriolysis of the root nodule organism would occur under field conditions. For this and other reasons, Vandecavaye and Katznelson believe that the experiments to date indicate only that bacteriophage *may* be responsible for these reduced yields of alfalfa on certain soils, but definite conclusions await further work.

MECHANISM OF SYMBIOTIC NITROGEN-FIXATION

For many years attacks on the important problem of mechanism of biological nitrogen-fixation were distinguished by few experiments and much speculation. Although it was realized that elucidation

tion of how microscopic organisms are able to bring about this important synthesis at ordinary temperatures and pressures may have far-reaching implications not only for this particular process but for solution of some of the mysteries of enzymatic and inorganic catalysis in general, progress hardly reached the stage of even definitely planned experiments. In recent years, however, several distinct types of studies have been undertaken at different experiment stations, the results of which provide experimental bases for discussion of the mechanism. Even if present interpretations are in error, the existence of views *based on experimental findings* rather than on unsupported speculations constitutes an advance since these views should stimulate the research necessary for correction or verification of the present hypotheses.

Carbohydrate-Nitrogen Relation

An ultimate aim of biological research is to provide mechanisms in terms of physics and chemistry of phenomena which have been previously restricted to empirical description. Little progress toward realization of this goal has been made on the root nodule problem until the recent demonstration that the carbohydrate-nitrogen relation in the host plant may condition certain responses associated with the fixation process. The value of this concept as an aid toward an understanding of the course of microbiological processes in the soil has long been appreciated, and more recently workers in plant physiology and horticulture have found it profitable to include consideration of this relationship in interpretation of some responses of the plant to its environment. Application of the *physiological* concept to symbiotic nitrogen-fixation, *i.e.*, that the carbohydrate-nitrogen balance in the host may act as a regulator for growth and fixation processes instead of the more literal idea that certain responses are related to supply of carbohydrate and others to nitrogen, was delayed until experimental methods were available which would allow controlled changes in the carbohydrate-nitrogen relation of the host by use of widely differing methods. During recent years a number of new techniques have been applied which overcome this deficiency; these include: (1) change in pCO_2 of the atmosphere; (2) change in pN_2 of the atmosphere; (3) intensity and duration of light; (4) addition of carbohydrate or of nitrogen; (5) variations in inoculation of plant with respect to

strain of organism used and time of application of bacteria (36, 122).

On the basis of evidence obtained from numerous experiments in which these techniques have been used, it was possible to develop a hypothesis (117) ascribing a regulatory mechanism in symbiotic nitrogen fixation to the balance between carbohydrate and nitrogen in the host plant as contrasted to the older views which emphasized the effect of carbohydrate or of nitrogen with little reference to their relationship. Since the literature review as well as the experimental bases for the hypothesis have been recently summarized (8, 117), only an outline will be given in this paper. A "normal" plant is defined as one growing under certain arbitrary environmental conditions, and other plants are classified as to their carbohydrate-nitrogen relation when referred to the normal plant as the standard. Associated with each of these classes are definite responses concerned with the nitrogen fixation process among which may be cited: (1) reaction of nitrogen-fixing system to combined nitrogen; (2) effect of strain of organism on size and position of nodule; (3) response of plant to changes in environment, e.g., variation in pO_2 in atmosphere or of light intensity; (4) determination of *ineffectiveness* of a strain. The last example was discussed only briefly by Wilson (117) because of lack of experimental results, but more extensive studies by Raju (69) and Reid (70) have confirmed and extended the interpretation given in the bulletin.

Isolation of Intermediates

The classical method for investigation of the mechanism of biological processes is to attempt the isolation of definite chemical compounds which may be concerned in the reactions. Important as this method has been, especially in the study of fermentations by micro-organisms, its value has been greatly vitiated through uncritical application. Too many investigators are prone to view the finding of a compound in the substrate in which a reaction has occurred as unassailable evidence that that compound is concerned in the reaction, especially if it can be fitted into an *a priori* hypothesis. The careful and often arduous experimentation necessary to prove that the compound is actually a part of the mechanism is seldom undertaken.

Because of the apparent simplicity of the reaction involved, for-

mation of ammonia as the initial step in the process has ever appealed to investigators of biological nitrogen fixation, but evidence in support of this view has been extremely unimpressive. Winogradsky (127) found that excised nodules liberated ammonia even in the presence of antiseptics or after drying at 40° C., but little ammonia was evolved from the roots of legumes free of nodules or from roots of non-legumes. Liberation of ammonia from nodules removed from the plant is not unexpected because of the high level of soluble nitrogenous organic compounds present including such free amino acids as asparagine and arginine which readily decompose to form NH₃ (66, 111). Burk (27) has examined a similar claim of Winogradsky concerning the significance of finding NH₃ in cultures of *Azotobacter*, and his conclusions with respect to its origin from breakdown of cell protein appear to be equally applicable to that found in excised nodules. Winogradsky's (127) arguments against this explanation are not convincing; moreover, no evidence has been presented to show that the nodules examined were actually fixing free nitrogen.

Of greater interest, because of the unusual nature of the experiments upon which it is based, is the recent proposal of Virtanen that hydroxylamine is the initial product of fixation. As early as 1910, Lipman of the New Jersey experiment station published an account of experiments which indicated that certain leguminous plants could provide soluble nitrogen compounds to non-legumes grown in association with them. In 1912 a more extensive account was given which provided definite evidence that under certain conditions uptake of nitrogen by non-legumes grown with legumes did occur, but because of the irregularity of the data, it was difficult to determine the origin of the benefit to the non-legume grown in the associated culture. Confirmation of the greenhouse studies of Lipman did not appear and the results of field investigations were indecisive. Various suggestions as to the source of the nitrogen obtained by the non-legume were offered including: (a) from non-symbiotic nitrogen fixation—the work was conducted in open pots and no special precautions taken to eliminate free-living nitrogen-fixing organisms; (b) from sloughed-off portions of the roots; (c) from nitrogen excreted by the nodules of the legume.

In 1927 A. I. Virtanen of Helsinki, Finland, unaware of the previous studies, reported that oats grown on a nitrogen-free sand in

open pots with inoculated peas developed as though they had been supplied with combined nitrogen. This initial observation was followed by quite extensive researches which have done much to increase knowledge of this interesting phenomenon. It was demonstrated that the benefit to the non-legume arose from nitrogen passed to it by the legume, since if the former were not present, soluble nitrogen could be detected in the substrate (102). In order to prove that this nitrogen represented compounds *excreted* from the nodules, detailed studies were made which eliminated the possibilities of origin from decomposing plant material or non-symbiotic nitrogen fixation. The following summarizes the proof on this question:

1. Nitrogen can be found in the substrate, or the indicator plant (non-legume) will show benefit in plant cultures grown under aseptic environment, *i.e.*, free of microorganisms other than the proper rhizobia (102). This eliminates the non-symbiotic nitrogen fixation explanation.
2. Nitrogen appears in the substrate as soon as fixation starts, which usually is too early for sloughing of plant tissue. Likewise, the quantities of nitrogen found in the substrate, or taken up by the indicator plant, may equal the total nitrogen in the leguminous plant; frequently 40 to 60 per cent of the total nitrogen fixed is found to have been excreted (100, 103). Such quantities are inconsistent with the view that the nitrogen represents sloughed-off tissue.
3. The nitrogen excreted in the substrate has been recovered and found to consist almost entirely of amino acids with traces of oximes (106, 109). In the earlier studies (109) the amino acids were believed to consist of equal quantities of aspartic acid and lysine, but recently (107) it was reported that β -alanine and not lysine was the second amino acid present. The origin of the β -alanine was apparently by decarboxylation of excreted aspartic acid by the root nodule bacteria in the substrate. If decomposition of roots or nodules were involved, many more amino acids as well as intermediates in protein decomposition should occur.
4. No excretion is observed from uninoculated leguminous plants supplied with combined nitrogen, which indicates that the nitrogen comes from the nodules (108).

Details of the extensive studies of Virtanen and associates will not be given since Nicol has provided three reviews of various aspects of the work containing excellent historical discussions with special attention to the significance of these findings for the widespread agronomic practice of mixed cropping (62, 63, 64). Virtanen likewise has published summaries of his experiments (98, 99). Although Virtanen's data are convincing and the description of the technique quite detailed, duplication of the results by other investigators has not been uniformly successful. Ludwig and Allison (58) made numerous experiments with a variety of leguminous species, but in no case were there evidences of excretion, even though in the majority of the experiments excellent fixation of nitrogen by the legumes was obtained. In the author's laboratory Bond (19) and Wagner (112) have likewise consistently obtained negative results. Their experiments were made for the most part with pea and oat mixtures though other combinations of legume and non-legumes were occasionally tested, but benefit to the non-legume was never observed. Also all attempts to detect soluble organic nitrogen compounds in the sand substrates in which inoculated peas, soybeans, alfalfa and clover had grown met with no success even though large quantities of nitrogen were fixed.³

Bjälfve (17) in Sweden also reported negative results. Thornton and Nicol (88) in pot experiments at Rothamsted investigated the influence of combined nitrogen on a mixed crop of alfalfa and rye grass and found more nitrogen in the rye grass alone than was added as NaNO_3 . Unfortunately, the experiment was not designed for study of the excretion problem so that the control of alfalfa and rye grass without NaNO_3 was not made until two years later. In this control, evidence of excretion was obtained after three months, but the quantity found was much less than in the presence of combined nitrogen (89). These experiments were made outside in open pots and were continued for several months; hence, origin of the nitrogen from non-symbiotic fixation as well as sloughing of plant tissue appears possible though not probable. Because of the practical importance of whether or not excretion occurs in the presence of combined nitrogen, repetition of the experiment under a more rigorously controlled environment appears desirable.

³ Through Professor Virtanen's courtesy the author recently carried out experiments in the greenhouse at Helsinki and secured positive results. The cause for the discrepancy between this work and our earlier negative experiments is not readily apparent and is now under investigation.

In spite of negative reports there can be no doubt, in view of the numerous positive results of Virtanen and co-workers, that excretion does occur under certain conditions, and that the quantity involved may represent an appreciable portion of the total nitrogen fixed. Whether this excretion represents a normal physiological process, or merely a response obtained under the artificial conditions of greenhouse plant-culture, constitutes the foremost question at present. To settle definitely this problem will require carefully designed field tests, but the undertaking of these appears inadvisable until the exact experimental conditions required for securing excretion are rigidly defined, so that duplication of the results can be made in any experimental station. Progress in this direction has been made by Virtanen and collaborators (100, 103, 105), and resolution of the difficulties may be expected in the near future. Results from the field experiments made to date have been unsatisfactory, but it must be remembered that these were carried out with little knowledge of the underlying factors and were accordingly premature. Field tests in America have, on the whole, given little evidence of benefit to the non-legume (37). Wartiovaara (116) in Finland reports positive findings with an oat-pea mixture in the field, but as his plots were not randomized, hence open to the objection of improper design, the data cannot be regarded as final. Also in his experiments separate yields of the oats and peas were not taken; the only significant result appears to be that the *percentage* of nitrogen in the oats was increased—a finding that does not necessarily mean excretion of nitrogen by the legume since in field experiments other factors may operate to bring this same response. Field experiments at Rothamsted, described by Nicol (64), were not designed to investigate the problem of excretion and as a result lack proper controls or analytical data. For this reason they shed little light on the question.

On the basis of finding that the excreted nitrogen is composed solely of lysine (later shown to be β -alanine from the aspartic acid) and aspartic acid with traces of oximes, Virtanen and collaborators (101, 106) propose that the first step in symbiotic nitrogen-fixation is formation of hydroxylamine (NH_2OH) which unites with oxalacetic acid to form the oxime of this acid and which in turn is reduced to aspartic acid. Oxalacetic acid is produced during respiration of carbohydrate according to a generally accepted mechanism

involving succinic, fumaric and malic acids; traces of fumaric acid were found in the substrate (106).

Although of interest, the suggested mechanism can be regarded only as an initial point for future research rather than as a final conclusion. Even though finding only two amino acids in the substrate is good evidence that these were *excreted* from the plant and did not arise from decomposition, it does *not* constitute equally good evidence that these compounds represent initial products of fixation. Unless the assumption is made that the fixation has occurred outside the plant in the rhizosphere, about the only definite statement that is possible concerning the origin of the two amino acids is that they are the only ones excreted. To state that they cannot be the result of protein breakdown in the nodule appears to be unwarranted. Nodules contain many free amino acids including arginine, lysine, histidine, tyrosine and asparagine (66, 111) concerning the origin of which there is equal doubt. The excretion of only one of the several amino acids known to be present in the nodule is certainly indicative of differential permeability to this particular acid, but to conclude that this permeability is necessarily connected with the fixation of elemental nitrogen appears to be only a gratuitous assumption.

Enzyme System

A hitherto neglected means of attack on the mechanism of symbiotic nitrogen fixation is the characterization of the enzyme system. Even though chemical compounds are isolated from the nodules or substrate, proof that they represent definite intermediates in the process is usually extremely difficult; hence, their presence may have little significance. On the other hand, even without definition of the precise chemical reactions, knowledge of the properties of the responsible enzyme system may allow some measure of understanding of, and control over the process. The success of Burk and co-workers (23) with the allied problem of fixation by *Azotobacter* has led to similar attempts at analysis of the symbiotic enzymatic mechanism through use of physical-chemical techniques.

Investigations (119) on the fundamental problem of relation of substrate concentration to activity of enzyme indicated that in red clover the fixation process is independent of the pN_2 in the atmosphere until the latter is reduced to .10 atm., at which level fixation

decreases with pN_2 . A Michaelis constant (concentration of substrate at which half-saturation of enzyme is reached) of the order of .05 atm. was indicated. This value is somewhat lower than the corresponding one for *Azotobacter* (.21 atm.) but is much higher than those usually found for reactions involving gases (23). Study of the pO_2 function revealed that assimilation of both free and combined nitrogen is independent of the pO_2 between .1 and .4 atm.; an increase in pO_2 above .4 or a decrease below .1 atm. cause nitrogen uptake of either the free or combined element to decrease. Such results suggest that molecular oxygen *per se* is not directly concerned in symbiotic nitrogen fixation, but other studies indicate that it is important in an indirect manner (121). Investigations on inhibitors gave evidences that hydrogen gas, usually assumed to be inert, may be a specific inhibitor for symbiotic nitrogen fixation. The mechanism of this rather unexpected type of inhibition is still undetermined (92, 123).

Rôle of Minerals in Symbiotic Nitrogen Fixation

Researches by Burk and others (15, 21, 22, 23, 26) have demonstrated that calcium (replacable by strontium) and molybdenum (replacable by vanadium) are directly concerned with nitrogen fixation by *Azotobacter*. Because of the obvious importance of determining whether or not the mechanism of the symbiotic and non-symbiotic processes are identical, similar studies on the rôle of mineral elements in nitrogen fixation by leguminous plants are desired.

For many years the beneficial effects of liming fields sown to legumes have been established, and it was assumed that the favorable effects were due to neutralization of soil acidity. Albrecht (1, 2) has investigated this problem by use of an ingenious technique in which calcium is supplied to the plants in the form of a colloidal clay. The original bases in the clay are replaced with hydrogen ions through dialysis and the resulting "hydrogen clay" is saturated to varying degrees with calcium hydroxide. Soybeans were grown in sand to which Ca-H clays of varying degrees of saturation were added so as to provide different levels of calcium at various pH values. Both nodulation and fixation of nitrogen rose with increases in either level of calcium or with pH, but quantitatively, calcium appeared to be more effective than decreasing acidity. In order to eliminate varying reaction and calcium levels

simultaneously, clays of a constant pH (6.9) were prepared which were completely saturated, partly with calcium and the remainder with other bases. These clays were added to the sand in such quantities that the amount of calcium per plant was constant, irrespective of how much of the saturation was due to calcium. Unfortunately, nitrogen-fixation in these experiments was very erratic, so the results are difficult to interpret, but when potassium or barium was used with the calcium, some correlation did appear in the degree of saturation of clay with calcium and fixation of nitrogen. When magnesium or methylene blue was used, however, no evidence of such a correlation was apparent; in the case of methylene blue this lack of response was believed to arise from difficulties in exchange between the dye and hydrogen ions excreted by the plant (3, 45). Albrecht (2) interprets these data to mean that calcium has a significance in symbiotic nitrogen-fixation similar to that demonstrated for fixation by *Azotobacter*. This conclusion does not necessarily follow since no combined nitrogen controls were included in the studies, and therefore it is impossible to state whether the effect of calcium is on the general development of the plant or only on the nitrogen-fixation function.

Examination of the possible rôle of the rare mineral elements in the fixation reactions has been restricted almost entirely to detection of these in the nodules (35). The observations of Konishi and Tsuge (50), that addition of small quantities of titanium salts increased nodulation of alfalfa grown in soil or agar and of nitrogen fixed in sand cultures, appear worthy of further study. Stapp (78) reports field experiments in which nitrogen-fixation by red clover and alfalfa was increased by addition of molybdenum salts.

Cytological Investigations

In contrast to non-symbiotic nitrogen-fixation, research on the mechanism of the symbiotic process includes fundamental problems which are independent of the chemical reactions involved. For example, answers to such questions as how the bacteria are able to invade, how they develop in the tissues, and how the fixed nitrogen is transferred to the host plant are of extreme importance for the understanding of the complete mechanism. Attacks on these problems have been provided by cytological and allied studies. A great share of such investigations has been made by Thornton and his

associates at the Rothamsted experiment station, and since he has recently reviewed these findings (85, 86), only an outline of the present interpretations of the data will be given:

1. The initial step in the invasion of the nodule is secretion by the plant of substances which attract the bacteria and stimulate invasion of the plant (Thornton, 1929). Lewis and McCoy (55) concluded that this secretion did not depend necessarily on the presence of the top portion of the plant, as was indicated by Thornton's results, since excised roots of the bean were invaded even in the absence of light. Etiolated intact plants, however, formed more nodules than did the roots alone; the presence of carbohydrate stimulated nodule formation on both excised roots and intact plants. That the stimulating substances are not excreted specifically by leguminous plants was demonstrated by the work of Ludwig and Allison (57) who concluded that the substances probably acted through stimulation of the development of the bacteria in the rhizosphere, either by furnishing accessory growth substance or food for energy. They also were of the opinion that the practical importance of these excretions in the field was negligible.

2. Deformation of the root hair or "curling" follows the initial attraction of the bacteria to the region of the plant. This curling apparently is caused by a secretion from the bacteria as filtrates of the cells will bring a similar deformation in absence of the living organisms. The curling is non-specific, *i.e.*, filtrates of cells from one cross-inoculation group will deform the root hairs on the plants belonging to another group (McCoy, 1932). According to Thornton (87), the secretion from the bacteria stimulates the development of root hairs with respect to both length and number, but this stimulation as well as deformation of root hair is checked in the presence of combined nitrogen. If dextrose is added with the combined nitrogen, the inhibitory effects of the latter on the development of the root hairs in the presence of the organisms or their secretions may be overcome. The interpretation of these observations is that the carbohydrate-nitrogen relation in the plant is an important factor in regulating the deformation of the root hair—the essential preliminary step to infection (87).

3. Even though a root hair is deformed, the probability that

it will be invaded is small, and even smaller is the chance that a nodule will develop (McCoy, 1932). If a nodule is formed, its growth may be adversely affected through addition of combined nitrogen; numbers of nodules are less markedly affected than is their size (44, 90). Cytological studies on nodules of the alfalfa plant grown in the presence of combined nitrogen as reported by Thornton and Rudorf (91) showed that the cell walls of the distal cap become much thicker than those noted in the normal nodule. Also the endodermis surrounding the central tissue of the nodule as well as that enclosing the vascular strands became abnormally thickened through the deposition in the cell walls of a substance giving the suberin reaction. Accompanying these changes in the structure of the nodule was increased necrosis of the infected tissue together with a tendency for the bacteria in the younger portions of the nodules to assume the coccus form, a stage in the life-cycle usually associated with food deficiency (85). It is of interest that other methods of producing lack of carbohydrate in the nodule, *e.g.*, absence of boron in the nutrient solution (Brenchley and Thornton, 1925) or by growing the plants in the dark (Thornton, 1930), also bring about necrosis of the nodule with the bacteria becoming parasitic on the plant cells, a condition that normally occurs in nodules only when they grow old or toward the end of the growing season, *i.e.*, under conditions that favor deficient carbohydrate supply (85, 86).

The mechanism by which the nodules transfer nitrogen to the plant has ever proved a fascinating subject for speculation. Four hypotheses have been advanced: (a) lysis by plant enzymes; (b) lysis by bacteriophage; (c) autolysis of the bacteria after death; (d) excretion of soluble nitrogen compounds coincident with fixation. Cytological investigations of the lupine nodules led Shaede (75) to conclude that the first hypothesis is correct; the data of Thornton, however, would in general support the fourth. Korsakova and Lopatina (51) found no proteolytic enzymes in the nodules of the lupine and think that Shaede's view is improbable. Bond (18), on the basis of experiments concerned with the rate of transfer of nitrogen from the nodule of the soybean to the remainder of the plant, claimed the data supported the fourth hypothesis; but Wilson and Umbreit (126) challenge this conclusion on the ground

that the data could be used with equal facility in support of the other views.

Little is known regarding the active agent in the fixation. At one time the bacteroids were believed to be responsible, but at present their viability is questioned since there is no evidence that they are able to reproduce (13). It should be kept in mind that the bacteria themselves do not have to constitute the agent which fixes the nitrogen, since the possibility remains that the nodular tissue, composed of both cells of the plant and bacteria, may bring about a reaction that neither component alone can achieve.

In view of the pronounced interest in accessory growth factors for both bacteria and plant, it is not surprising that suggestions are made that these play a rôle in the symbiosis. McBurney, Bollen and Williams (59) showed that the bacteria from alfalfa produced pantothenic acid and that the nodules of alfalfa were a good source of "co-enzyme R." Since in short-time experiments addition of purified pantothenic acid increased the dry weight (but not the nitrogen content) of uninoculated alfalfa plants about as much as did formation of nodules, they concluded "considerable part of the stimulating effect of the bacteria is due to the pantothenic acid synthesized and excreted by the organism in the nodule and passed on to the plant." It should be noted, however, that these experiments were carried out under highly artificial conditions, *i.e.*, agar substrate in cotton-stoppered tubes. Whether under natural conditions in the soil, which is an excellent source of both pantothenic acid and "co-enzyme R," the mutual interdependence of host and bacteria for accessory growth substances would play a significant rôle in the symbiosis is open to question. Likewise the increase in dry weight of the nodulated plants may have come from the small quantity of nitrogen fixed rather than pantothenic acid synthesized; without a combined nitrogen control the origin of this increase is obscure.

Thimann (79) proposes that the cause of nodule formation is the production of auxin (indole acetic acid) by the bacteria—probably from tryptophane in the nodule. The auxin stimulates cell division but prevents cell elongation so that a shapeless mass of parenchymatous tissue results. This idea has attractive possibilities but will need support by further experiments such as an actual demonstration of production of auxin by the bacterial cells and evidence

that secretions from the root nodule bacteria will cause a response similar to that obtained when auxin from other sources is used. As previously reported, the chief reaction to filtrates from the bacteria has been root curling without formation of anything resembling a nodule.

An interesting observation made by Laird and West (54) is that hypocotyls of red clover seedlings grow upward in a vertical position if sprouted on a seed bed containing crude Bios 2, the growth stimulant for yeast and bacteria. This reaction is confined to primary stages of seedling growth since secondary roots develop normally and penetrate the substrate. It was demonstrated that the factor responsible for the reaction is Bios 2b alone, for Bios 1, Bios 2a, pantothenic acid and various amino acids were inactive with respect to this root-bending; heteroauxin which is chemically distinct from Bios 2 produced a similar but not identical form of root bending.

In summary, it is apparent that encouraging progress has been made from several distinct points of view toward solution of the problem of the mechanism of symbiotic nitrogen fixation. There is danger, however, that much uncritical thinking in analysis of the results may take place unless care is taken to appreciate the difficulties of the problem. Burk (24) stresses the necessity of employing the most rigid standards of logic in evaluating experimental data concerned with mechanisms and suggests that satisfaction of certain criteria be required for the acceptance of any proposed chemical path or mechanism.

Criticisms of much of the published work dealing with the mechanism of both symbiotic and non-symbiotic nitrogen-fixation can frequently be made not so much from the point of view of actual disbelief in the conclusions, but rather doubt that the data submitted will support the broad and sweeping generalizations made. Unless the findings are constantly reviewed in the light of some such rigid standards as those proposed by Burk, confusion rather than understanding may prove to be the ultimate reward for our labors.

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Since completion of the manuscript two papers of interest for certain portions of this review have appeared. Clark (Cornell Univ. Agric. Expt. Sta. Mem. 196) reports negative results with

respect to nitrogen-fixation by the organism apart from the host plant when tested under a wide range of experimental conditions including use of "resting cells" (mass cultures), presence of other organisms, addition of growth stimulants, and increase in the pN_2 . In the same memoir he describes the chemical properties of the accessory growth substance (Coenzyme R)—and in contrast to Thorne's findings states that continuous cultivation of the organism on media made from purified chemicals with NH_4Cl as the source of nitrogen is not possible without addition of the growth factor. It is suggested that differences in the quantity of inoculum used may be the origin of the discrepancies in the results of the various workers with respect to the essential character of the growth substance.

Verner and Kovalev (Compt. Rend. Acad. Sci. U.S.S.R. n. s. 4: 325, 1936) claim that the root nodule organisms are able to fix free nitrogen alone if furnished with "Bios" from yeast or young pea or carrot plants. As this is the first paper in several years in which evidences of fixation have been presented, verification by other workers is desirable, especially since addition of extracts which probably contained "Bios" were without effect in the experiments of other investigators (Allison, 1929; Lohnis, 1930; Pohlman, 1930, 124). The use of more purified extracts essentially free from nitrogen, however, may be the decisive factor in these experiments.

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SOME FUNDAMENTAL PROBLEMS OF TAXONOMY AND PHYLOGENETICS

KNUT FÆGRI

Bergen, Norway

When asked by the editors of *The Botanical Review* to contribute an article on "Taxonomy or phytogeography of northern Europe," it seemed very natural to deal principally with those phytogeographical investigations of the Scandinavian¹ flora which have attracted so much attention during recent years. However, I have also wanted to include investigations on some of the fundamental concepts of taxonomy, for the phytogeographical reasonings are naturally much influenced by these fundamental concepts. This article is to be considered, therefore, as a theoretical introduction to the problems with which I shall deal in my next contribution. This is the reason why I shall be concerned primarily with the opinions of Scandinavian authors, though these problems are, of course, of a general nature and though much work has been done on the same subject in other countries.

THE SPECIES PROBLEM

Both geneticists and taxonomists in Scandinavia have contributed greatly during past years to the discussion about, and the solution of, the species problem. It cannot be denied, however, that these two groups of botanists have worked rather independently, and that cooperation between them has been absent to a great extent. The underlying reason for this is not, I think, so much a matter of difference of opinion about the species concept itself, as it is a matter of difference in the way the whole problem is approached. The geneticist starts with a supposedly homogeneous population and looks for differences, whereas the taxonomist starts with a supposedly heterogeneous population and looks for similarities. The entire mental adjustment, consequently, is totally different, and even if the two groups work toward the same goal, they approach it from different directions and are not in

¹ Throughout this and a subsequent article I shall, for the sake of brevity, use the term "Scandinavia" as comprising Denmark, Finland—Suomi, Norway and Sweden, together with the adjacent parts of U.R.S.S.—Kola and Karelia.

contact with each other along the way. This is an important reason why taxonomists and geneticists have polemised so much about this subject, even though the real differences of opinion are relatively insignificant.

The most comprehensive contribution to the discussion of species, genus, family, etc., concepts lately given by Scandinavian taxonomists, is that of Du Rietz (1930a), to which I can only refer here. In his species definition, Du Rietz (p. 357) lays special stress upon *discontinuity* as the only criterion which can be used to distinguish between species in sexual and asexual populations alike.

This paper of Du Rietz has been rather severely attacked by Müntzing, Tedin and Turesson (1931) who claim that Du Rietz over-rates the purely geographical and under-rates the genetical point of view. This objection is hardly quite justified, for in his species definition Du Rietz (p. 358) expressly states that species must be genetically separated from each other. Geneticists sometimes claim that genetical experiments are necessary for the determination of species limits, and that the species concept should be purely genetical. There are many reasons why this cannot be accepted by taxonomists, and one very simple reason I may mention here: The thorough taxonomic-genetical analysis of a genus, of its inner structure and relations to other genera—which has, so far, not been accomplished in a single plant genus, with the possible exception of *Anthirrhinum*—is, if not always the work of a lifetime, at all events a work of many years' intense studies; in the case of trees, perhaps a work of centuries. One can hardly expect taxonomists and phytogeographers to wait so long! Besides, it seems that the taxonomist is usually able to define species limits quite well without the aid of the geneticist. And in dubious cases the geographical methods, which are essentially foreign to many geneticists, often settle the question. Intending, by no means, to under-rate the genetical point of view, for the geneticists' contributions to the knowledge of the inner structure of the species has been of fundamental influence, I cannot accept the *genetical experiment* as the master key, the one and only master key to taxonomy. I shall later return to this question.

Very important contributions to the discussion of the species concept have been given by Turesson who has advocated his

opinions in a series of papers (1922-1936). Turesson's ideas have constituted a central point of present day discussion about species problems. He has called his theory *genecology*; one might call it the theory of the genotypical response of the plant species to the habitat; popularly, it is perhaps best known as the doctrine of the *ecotype*. As this doctrine is now quite well known among botanists, I shall not deal with it in detail here; it might suffice to mention, however, that, according to Turesson, the species reacts upon the influence of the environment in such a way that a special hereditary form, an ecotype, is formed in each habitat. The ecotype is to be considered a product "arising through the sorting and controlling effects of the habitat factors upon the heterogeneous species population" (1925: 147). Even if I do not think it has been expressly stated, it is generally assumed that ecotypes are compatible. Cases of incompatibility, especially those conditioned by different chromosome number, will not be treated as ecotypes in this article. The existence of ecotypes has been proved in a great number of cases, lowland types differing from alpine ones, coast types from those of inland habitats, etc. Turesson has always maintained (1936: 422) that the variation of biotypes within a species is (at all events, in most cases) discontinual, the biotypes from similar habitats resembling each other closely and forming together one ecotype, while those of other habitats are plainly different and constitute other ecotypes.

On the other hand, Langlet (1934, 1936), from a very close study of the variation of a very great number of proveniences of pine (*Pinus silvestris* L.) all transplanted (sown) to experimental fields, concludes that the contents of dry matter (a very important character, of which frost resistance, etc., show a decided dependence) is a simple function of the number of days with a temperature $\geq 6^\circ$. This and other evidence Langlet considers to be a direct demonstration of the continuity of variation of ecotypes (proveniences).

His opinions have been strongly disputed by Turesson (1936: 425) who maintains that the investigations of Langlet are genetically dissatisfactory, as young first generation plants have been used for investigation. On the other hand, Langlet points out (1936: 377) that the number of biotypes studied by Turesson is too small to permit any conclusion about continuity or discontinuity of variation.

As habitats do vary continually, and as ecotypes are, according to the theory, produced by the selective forces of habitat factors, a continual variation is, in fact, included in the ecotype definition for all species with a continuous area of distribution, unless one takes reservations that the difference between two habitats must attain a certain minimum value before it has any selective effect. This has not been done and seems rather improbable.

As it is, another possibility should be mentioned. If a species is distributed, *e.g.*, from the lowlands into alpine regions, but for some reason is scarce or lacking in the sub-alpine region, the species in question should have two mass-centra. In these mass-centra the species may be represented by widely different ecotypes which are very clearly distinct or connected by a very scarce series of intermediate ecotypes only. The same reasoning may be applied, of course, to seashores, limestone crags or other habitats. In such cases, there is a discontinuity of variation, but this discontinuity is a purely *statistical* phenomenon only, not connected with the nature of the genetical response of the plants to the habitat. In species having a continuous distribution this kind of discontinuity is not to be expected.

If discontinuities occur where, according to the theory of genetical response to habitat, variation should supposedly be continual, such cases should be taken up for thorough examination to detect the reasons for this behaviour. Such cases are those where intermediate habitats are inhabited, not by intermediate ecotypes, but with a mixture of both ecotypes, as repeatedly described by Turesson (1922: 331; 1925: 197). If response to habitat factors alone formed the ecotypes, there is no reason why an intermediate habitat should not produce an intermediate ecotype just as extreme habitats produce extreme ecotypes.

The whole problem of the continuous or discontinuous variation of biotypes is not ready for settling; it ought to be taken up for thorough investigation, however, for it is the fundamental question of genecology. It is especially important for the relations between genecology and taxonomy. If there exists a direct response to habitat factors, the variation will most probably be continual and the number of ecotypes, consequently, practically unlimited; there will then hardly be any reason to include ecotypes in taxonomical handbooks. If, on the other hand, variation is

discontinual and the number of ecotypes limited, they will certainly be a very valuable addition to taxonomy and even more so to ecology and phytosociology.

Another problem, which should also be taken up for investigation, is the question of the *stability* of ecotypical characters. Turesson has entered upon this point repeatedly, but only indirectly, and it seems that he, at all events in his later papers (1922: 339), presumes a very great degree of genetical stability; a species, *i.e.*, all biotypes together forming the species, has a certain genecological potentiality, a certain stock of genes, of which, by action of the habitat, a certain combination is realized in each habitat and the rest of the genes are more or less completely lost (Turesson 1925: 228; 1927: 93). The biotypes occurring in the extreme habitats have, therefore, according to this view, lost most of their original stock of genes through the selective forces of the habitat and cannot give origin to less specialised forms.

I must confess that I have great difficulty in accepting this view which is certainly in accordance with the prevailing genetical theories but which absolutely does not fit in with the known facts of evolution. We may imagine a species as having come into existence somewhere in the lowlands. Some of the biotypes are capable of surviving in the alpine region; in fact, they are "adapted" to alpine conditions, and during the wandering of our species toward the alpine region, the other (lowland) biotypes are gradually eliminated by selective forces of the habitat, until the alpine ecotype alone survives. But how can an alpine ecotype come into existence in a lowland habitat? It must necessarily be eliminated by selective forces of the lowland habitat while still in *statu nascendi*. The same is, of course, the case with the "alpine genes" taken singly. One might rather imagine that migration of the species into the alpine region and the origin of alpine biotypes are totally dependent on each other and absolutely simultaneous. Which, then, is the cause and which the effect, I shall not enter upon.

In consequence of his view on genotypical stability of ecotypes, one should expect Turesson to adhere to the gene-center-theory of Vavilov, that each species has a gene center at its place of origin, the number of genes gradually diminishing toward the periphery of the distribution area. In a special paper (1932a),

Turesson has pointed out, however, that the theory of Vavilov is in some cases actually not in accordance with geological facts, and he assumes that climatic changes during the quaternary period brought about migrations of biotypes, which should have the effect that the gene center is to be found in a place where climatic conditions are similar to those of the place and time of origin of the species. If we accept the idea of absolute genotypical stability of ecotypes, such a theory is certainly the only way to dispose of the problem, which is, however, much easier explained in another way, as we shall see.

Schematically, the "life history" of a species might be formulated thus: At first it consists of a genetically and morphologically rather uniform population within a restricted area, *i.e.*, with small *actual* polymorphism. The *potential* polymorphism is very great, however, as pointed out by Du Rietz in the paper mentioned (1930a: 401), and the species gradually spreads from its center of origin, realising the morphological and ecological possibilities of variation. This is the first phase.

Next comes a period when the area of distribution has grown so extensive, geographically or ecologically, that a free intercrossing throughout the whole population is impossible. If the different parts of the population are subject to different external conditions, as they always will be, the selective forces of the habitat then induce a certain differentiation within the partial populations. The same would most probably be the case even if the external conditions were identical, as each population would mutate in its own way. Still, these partial populations are compatible and cannot be considered specific units. This is the second phase, the stage of ecotypes².

As we know from geologic evidence that differentiation is always going further and new genes are coming into existence, the third phase in the development of our species must be a stabilisation of the differences. In other words, our ecotypes must develop into

² The isolation between forms may be ecological as well as geographical; it may even be chronological if the populations have different flowering seasons. A very interesting example of this has recently been described by Iversen (1936: 85). Such cases, which we might call chronotypes, must not be confused with those of seasonal dimorphism which are connected with differences in the number of chromosomes (Fagerlind 1936), as such forms are probably incompatible, nor with the case described by Müntzing (1931) where the second generation is formed simply by the innovation shoots of the first one.

new species by the formation of incompatibility barriers between ecotypes developed from the same original stock. So far, I think our account is in accordance with that of Du Rietz (1930a: 352; 392). But on one point I cannot agree with the exposition given by Du Rietz; the "law of differentiation by means of automatic reduction of the potential polymorphism," originally formulated by Hagedoorn, must be compensated for in some way—if not, the whole mechanism of evolution would have stopped at a very early point, because all power of differentiation would have been exhausted. If the partial populations did not change any more, they would certainly soon pass over into the class of rare endemics which are on the verge of extinction.

If they are going to make the base of further development, the new species must have the power of differentiating into new ecotypes, these into new species, and so on. The reduction of potential polymorphism of the original species must be compensated for by a corresponding increase of potential polymorphism, *i.e.*, by further power of differentiation in at least some partial population.

The whole process of species formation is, of course, not schematically simple and divided in separate phases like this example. When, for example, *Chamaenerium angustifolium* or *Melandrium dioicum* has decided gene centers in Scandinavia (Turesson 1932), I think it is quite unnecessary to ascribe them to wanderings of the original gene center; I should be inclined to think that these partial populations have retained a greater power of differentiating, while the extra-Scandinavian ones have lost this power. In other words, before the said species have reached the third phase, a new development, phase one, has begun. I think we may consider the gene center rather as an indication of future development of the species than as a relict of the past history, as has been done by Vavilov and Turesson. The location of such gene centers is hardly in any way connected with the location of the center of origin of the species; the existence of such centers of variability seems to depend wholly upon those unknown factors which determine the differentiability of the species.

We are here at a fundamental point: What determines whether or not a population shall develop further, expand and differentiate, or whether it shall keep its habitat as a specialised, mono-ecotypical form until obliterated by competition or by geological changes?

Why do some groups, genera or families have a seemingly unlimited power of differentiation, while others, even orders and classes, seem to consist chiefly of quite stable and constant units? Why do the large groups of the vegetable kingdom show periods of intense differentiation and development, followed by periods of evolutionary senility? These are fundamental questions which, unfortunately, still belong to metaphysics, not to biology.

This highly hypothetical excursion has been an attempt to find the position of the ecotypes in species development, as a first stage in the differentiation process, and we shall now return to the species concept once more. A principal reason for the present, somewhat confused state, certainly is to be found in the extraordinarily varied claims which are laid to this concept. The species shall be the common fundamental unit of taxonomy, genetics, cytology, chorology and phylogenetics; consequently, taxonomists, geneticists, cytologists, phytogeographers and phylogeneticists all present their claims. Everyone tries to form a species concept according to his own requirements, resulting in the present confusion.

The taxonomist is rather easily satisfied, the taxonomical species concept being: those individuals which have certain, essential characters in common.

The geneticist demands for a species a group of biotypes, isolated from all other biotypes by incompatibility barriers.

The phytogeographer possibly accepts the taxonomists' concept, but adds some geographical reservations, and the cytologist demands a common chromosome garniture. (Certainly in the cases of cytologically irregular species, the prevailing species delimitation is usually accepted.)

Thus, each branch of botany has its own, easily definable species concept, which does not presume any validity outside that branch. If we now try to go one step further and find a species concept that is valid for all branches, a *general* species concept, difficulties arise: No single method has been able to provide the certain criterion of the nature of the "general species" (cp. analysis in Stojanoff 1936). Consequently, one has been obliged to abandon that idea and has tried to combine the demands of the different branches, and instead of a *general* species concept, we are in possession of a number of *combined* species concepts, which is not

necessarily the same. To me, one of the best proofs of the existence of the species as a natural unit is that all these highly incongruent demands can really be combined with some success. But even if the overwhelming number of species are delimited in the same way by all criteria, there are some groups, genera or parts of genera, where delimitation according to, *e.g.*, the taxonomical concept, does not accord with that of, *e.g.*, the genetical. If we try to apply our combined species concept in such a case, we find that delimitation according to one of the criteria is narrower, or wider, than that according to another criterion. In those cases the most reasonable thing to do is possibly to discard the whole "general species" idea, as one cannot expect nor force the taxonomist to accept a species delimitation which is essentially non-taxonomical nor, even less, a geneticist to accept one that is non-genetical. In such cases, each branch of botany must have a fundamental unit of its own and possibly call it a species. I do not think that this should lead to any confusion if one does not try to use the units in the wrong places.

And even if the demands of taxonomy, genetics, *etc.*, might still be combined into one species concept, other and even worse difficulties are met with when the phylogenetical concept is taken into account—and nowadays perhaps most of the papers published on taxonomy, morphology, genetics, *etc.*, have a phylogenetic aim. Phylogenetically, the species is the momentary realisation of a line of evolution (Fægri 1931, 1935a). This is rather perplexing as it in reality implies that all demands for constancy, for congruence with a given type, must be left out. This is, however, even if not usually realised, an inevitable consequence of our accepting the evolution idea. It involves that a species may change and still remain the same species so long as the line of evolution is intact; if the line of evolution branches, however, *none* of the new species formed by this process is identical with the former species, even if one of them should happen to resemble that more than the others, because none of them is now an expression of the same line of evolution. Here is not the place to enter upon all consequences of this phylogenetic species concept, nor upon the facilitation which it represents in phylogenetical reasonings. I must refer to my previous publication (1931) and even more to Lam's paper on the phylogenetic symbols (1936), especially his fig. 32, which also in

a most striking manner illustrates the "life history" of a species given above.

One might object to Lam's illustration that it does not account for those cases when a species arises suddenly, not through a gradual development of an existing line of evolution, *e.g.*, through autopolyploidy or chromosome doubling after hybridisation. Such cases of sudden species formation are certainly rather rare. Another objection should possibly be made with regard to polyphyly (or polyrhieity, as Lam has called it, reserving the term polyphyly for the absolute concept, *i.e.*, polyphyly based upon different acts of creation) in species formation. I should think that within a relatively narrow unit, like the species, polyrhieity cannot be expected, but in such cases where partial populations are mixed before final genetical stabilisation, which should hardly deserve the designation polyrhieity at all. In the case of genera, and even more of higher units, polyrhieity is certainly rather usual and more frequent than in the case of species.

Phylogenetically, the species is thus no fundamental unit, the fundamental unit being the line of evolution. I can now easily imagine the reader objecting that the definition given above is too vague—what is a "line of evolution," which are the criteria of such a line? This question means, what is the taxonomical, genetical, *etc.*, meaning of the term—as the phylogenetic meaning is obvious—which are the taxonomical, genetical, *etc.*, criteria. I have, therefore, tried to give a "combined" definition (1935a): A phylogenetic-taxonomical line of evolution is a sequence of generations, the individuals of which descend from the individuals of the preceding generation and within each generation arrange themselves according to the law of probability with regard to all essential features, and, further, form a closed sphere of combinations, reacting avitally or incomparably with all other spheres of combinations with which it comes into contact.

I want to draw attention to the last point: "with which it *comes* into contact." One cannot know if some geographically, or in other ways, isolated population does not in reality belong to the same sphere of combinations. If they do not come into contact with each other, no hybrid formation takes place in nature, and there is no reason for invalidating the species. If, on the other hand, the two populations are really brought together and hybridise

freely and the "species are more or less lost in an overwhelming majority of highly polymorphic hybrid-populations" (Du Rietz 1930a: 381), I cannot see any reason for keeping the original species as separate units. This case has been discussed rather thoroughly by Du Rietz (l.c.) who maintains that even if the procedure might be the only genetically satisfying, the results should be, in the case of *Salix*, "few and extremely compound species, very difficult to handle and each consisting of so extremely different forms that they would be of little value as units for ecology and all the other branches of botany that taxonomy has to serve" (p. 385). Certainly, one should not throw away the former units, but keep them as subspecies or whatever one might want to call them; at all events, I should not like to accept such forms as species in a phylogenetic sense. They do not represent different lines of evolution; the effects of any factor, internal or external, influencing one of these forms, are through the free intercrossing distributed all over the whole population. Most probably such cases as these represent species in *statu nascendi*, i.e., lines of evolution in the moment of dividing, but they have not divided as yet, and as we cannot to-day predict how the results will be when the division is accomplished, we might just as well resign.

Usually such very polymorphic syngameons are treated as consisting of some "pure" species and a large number of hybrids. Sometimes, the "pure" species might be extremely rare and known in a few specimens only. The power of free intercrossing shows that these "species" must be closely related and have a common ancestor; in other words, we have here a species development that has never reached the previously noted third stage in our "species life history." Why should such forms then be treated as species any more than other ecotypes? That they are morphologically more different does not seem to be a good argument.

If it is for taxonomical or phytogeographical or any other reason desirable to divide such an "extremely compound" species into smaller units, this must of course be done. These smaller units may possibly even be designated as species in a taxonomical sense, not phylogenetically or genetically, but I should prefer to call them something else, subspecies or even varieties. One question which is very important, not only to ecologists but even more so to quaternary geologists, is the question which was put forth by

Wesenberg-Lund (1906 and in Johansen 1906), namely, as to whether or not the morphologically identical forms are also ecologically identical. This is far from certain, even if it is very probable, as ecologic conditions will influence phenotypical realisation of characters as well as, through selection, the genotypical composition.

The opposite extreme of these morphologically very compound species is the case of morphologically identical forms (number of chromosomes included) which are incompatible for some physiological reason. Such forms must be considered as belonging to the same taxonomical species; taxonomically, it is not possible to distinguish between morphologically identical forms. This opinion was maintained also by Clausen (*cp.* below). Such incompatibility should occur if a species were polyrheitic. I do not think that any case of absolute intraspecific incompatibility has been demonstrated in which a species is divided into parts which are incompatible with *all* other parts of the same species (cases of autoploid excepted, which I consider separate species), as should be expected if the species were polyrheitic. Such partial species should, irrespective of their origin, belong to different lines of evolution, as no gene exchange can take place between them. And they should certainly part very soon since lack of intercrossing should favor differentiation. Partial intraspecific incompatibility, as known in a great number of cases, is, of course, of no importance in this connection.

A rather intriguing case of morphological identity is represented by some of the ecotypes described by Turesson (1922b, 1931b). In certain habitats all forms of a given species were identical, but on transplanting to experimental plots it proved that some of them did not change; they represented a special ecotype, while others changed back to the "normal" form; they were just modifications. If the two types were really absolutely identical, this is a case where the genetical demand for distinction between genotypically different forms is absolutely contradictory to the taxonomical demand that morphologically identical forms cannot be separated.

As two individuals are never absolutely identical, the term "morphologically identical" is only relative, referring to the essential characters and to a variation of these according to the laws of probability. Which characters are to be regarded as essential and

which as not, is and must always be left to the investigator to decide. The practical species *delimitation* is, therefore, always somewhat arbitrary, but this arbitrariness does not affect the species *concept*; it is a practical difficulty only. In amphimictic populations, the problem of the microspecies is certainly not too serious.

With regard to amphimictic populations, Turesson has introduced the terms *coenospecies* and *ecospecies* as units superior to the ecotype. These units have been repeatedly defined; in the joint publication with Müntzing and Tedin (1931), the coenospecies is stated to consist of "all individuals capable of gene exchange by direct or indirect crossing." This is in many cases a very comprehensive unit, and in nature it is divided into smaller groups, separated by "more or less sterile or unviable hybrid populations." These smaller biotype groups, consisting of one or more ecotypes, are those which are called ecospecies. Usually the ecospecies corresponds to the well known Linnaean species.

Accordingly, the coenospecies includes biotype groups between which the sterility barriers are pronounced, but not absolute. Even if this unit is in some cases of immediate taxonomical value (*Erophila verna* coll. is quoted as an example, Turesson 1931b) it must be feared that when *all* possible combinations with *all* related forms, indigenous and foreign, have been tested, the coenospecies may become too extensive to correspond to any *species* concept. It may then be considered a purely phylogenetic unit, the capability of gene exchange being taken as a sign of common origin (*cp.* Turesson 1929: 333). However, sterility barriers are sometimes found in the most unexpected places, and a form not belonging to the coenospecies may, nevertheless, be phylogenetically very closely related to some part of it, even more closely than the different parts of the coenospecies between themselves. Müntzing has pointed out (1933) that closely related species may be incompatible for purely quantitative reasons, autopolyploids often reacting incompatibly with the corresponding diploids.

Where hybridisation is conditioned by chromosome doubling and the amphipolyploid is incompatible with one or both of the parents, gene exchange does not take place. Such plants do not belong to the same coenospecies.

At the 19th Scandinavian Congress of Natural Sciences, Clausen

(1936) gave a very interesting account of the work done by himself and collaborators on species delimitation and species concepts in California.³ As it was a preliminary account only, and as an exhaustive publication has possibly appeared from the Carnegie Institution of Washington before this is read, I shall not enter upon the matter here. Clausen has had the opportunity of utilising the modern taxonomical and genetical concepts, e.g., the eco-type idea, in field work and experiments in cooperation between geneticists and taxonomists, and his results seemed very promising.

In the preceding account I have entered upon the species problem in amphimictic populations only; in apomictic or mixed populations, the problem is essentially different, but I shall not enter upon that here. Even if they are perhaps more frequent than usually realised, apomicts nevertheless play a much smaller part in nature than in some theoretical considerations. The case has been discussed by Du Rietz and Turesson, and the latter has introduced the term *agamospecies* to designate "an apomict-population, the constituents of which, for morphological, cytological or other reasons, are to be considered as having common origin" (1929: 333). The term is a handy warning that the form in question is not propagating in a regular way, but suffers from the same deficiency as the term coenospecies: both of them are too extensive; they are distinguished by the geneticist's desire to differentiate between groups rather than by the taxonomist's idea of combining individuals.

SPECIES FORMATION

Some years ago, Müntzing (1930b, 1932b) published the highly interesting results of a species cross in *Galeopsis*. *G. pubescens* and *G. speciosa*, both having the diploid chromosomes number 16, were crossed and gave a pronouncedly sterile F_1 . In F_2 , which consisted of some 200 individuals, one plant was found to be triploid. It was pollinated with pollen from *G. pubescens* and gave one seed which developed into a tetraploid plant with good fertility. The sensational feature in this case of amphiploidy is that both the triploid and even more its tetraploid daughter and the descendants of the latter are morphologically and, with the ex-

³ It is expected that Dr. Clausen will submit an article to the Botanical Review in the near future on this subject.

ception of the triploid, also genetically and cytologically, identical with *G. tetrahit*, as has been proved by close analysis of the synthetic form. The range of morphologic variation, which is rather wide in natural *G. tetrahit*, is even greater in the synthetic population, but this might be an effect of selection.

Müntzing concludes that the genotypical constitution of the synthetic plant must be: 2 *pubescens* genomes, 1 *speciosa* genome and 1 recombination genome. As natural *G. tetrahit* is genetically identical with the synthetic, this is the first case of a "good" species existing in nature having been synthesised from the genomes of two other species. It must be remembered, however, that *G. tetrahit* is a genetically somewhat irregular species, as a pronounced intraspecific sterility has been demonstrated by Müntzing (1929, 1932a). *G. tetrahit* is widely distributed in Scandinavia, chiefly as a weed, but its occurrence is by no means dependent on man, for the plant is also found in natural habitats and its seeds have been discovered in various strata of peat-bogs (Holmboe 1903: 190)⁴. *G. speciosa* is an exclusive weed, also very common in Scandinavia, but *G. pubescens* does not occur here. Statements to the contrary are wrong.

It is rather curious that, while the diploids, *G. speciosa* and *G. pubescens*, have large flowers and are insect pollinated, the tetraploids, *G. tetrahit* and *G. bifida* (which is closely related to *G. tetrahit* and has a somewhat similar genetical structure—one might perhaps suppose one *speciosa* genome more and one *pubescens* genome less?), have small flowers and are usually autogamous (Müntzing 1930: 186).

Later, Müntzing (1933) took up for discussion the general problem of hybrid incompatibility and the origin of polyploidy, and has demonstrated that auto- and allopolyploids are usually incompatible with the diploid species. This incompatibility preserves polyploids from being back-crossed to the diploids, which would break down polyploidy. He favours the theory that disturbance of the relations between number of chromosomes, i.e., amount of chromatin in the embryo, endosperm and mother plant, is the major reason why crosses between diploid and polyploid plants usually do not succeed. A main reason for the frequent occurrence of polyploidy in plants as contrasted with animals he sees in the oc-

⁴ My thanks are due Prof. H. Holmboe for pointing this out.

currence of double fertilisation and endosperm development in the former.

This view has been attacked by Heilborn (1934) who maintains that the theory of Müntzing does not explain the whole case. He advocates the opinion that several factors collaborate to produce sterility in such cases.

In a recent paper, Müntzing (1936) has developed his views further with special reference to the part played by autoploidy in species formation. Analysing 58 cases of autoploidy, the author concludes that polyploids differ from diploids in almost every respect and that these differences are such as to be of evolutionary value.

In many cases, "good" and well known northern European species have been proved to consist of one diploid and one tetraploid, e.g., *Empetrum nigrum* (tetraploid: *E. hermaphroditum*), *Vaccinium uliginosum*, *Campanula rotundifolia*, etc. The forms have a markedly different ecology, the tetraploids being usually "adapted" to the extremer habitat, as demonstrated by Hagerup (1932) both in the case of low (Greenland) and high (Sahara) temperatures. In our cases, the tetraploids are usually those going farthest to the north. This seems a little remarkable as Müntzing has demonstrated that "the negative correlation between chromosome number and rate of development is a universal phenomenon almost without any exception" (1936: 298). This would account for the behaviour of species like *Vaccinium uliginosum* and *Campanula rotundifolia*, where the diploid is found under the extremer conditions (Hagerup 1932, Böcher 1936), but not for a case like that of *Empetrum*. *Per se*, the slower rate of development must be a decidedly unfavorable character in places where vegetation periods are short. Consequently, one must suppose this unfavorable character to be not only compensated for, but over-compensated, by some other factor, as these slowly developing forms really maintain their places under those unfavorable conditions. The explanation for this is possibly to be found in the different chemical composition of the tissues (*cp.* the higher vitamin C contents of polyploid apples: Müntzing 1936: 367) which might increase resistance to low temperatures, drought, etc. In the Sahara, too, the slower rate of development of polyploids, if present, must *per se* be rather unfortunate on account of the short

duration of the rainy period. Very interesting is the case of *Eragrostis* related by Hagerup (1932). Thanks to wind drift, all three species occurring in the region are sown everywhere; but in the latter and drier part of the vegetation period, the species with 20 chromosomes cannot survive in the driest and hottest places; some time afterwards also that with 40 chromosomes dies and the octoploid, with 80 chromosomes, is the only one which accomplishes its life cycle in these habitats. In more favorable habitats the other species can also maintain themselves.

To return to Müntzing's paper (1936), this is not the place to enter upon particulars (*cp. Biological Abstracts* 10: 17673); the paper is in itself a review of the problem of autopolyploidy, and it is equipped with a very exhaustive list of references which should certainly prove to be of great value. Müntzing concludes that autopolyploidy must play a very important part in phylogenetic evolution, for autopolyploids are morphologically and ecologically more or less different from the forms with lower chromosome number. Important also is incompatibility, which brings about sudden genetical isolation in contrast to the case of ecotypes, etc. As such genetically isolated forms represent independent lines of evolution, I should be apt to consider them independent species too.

If an autotetraploid can be proved to be formed regularly when a species is exposed to unfavorable conditions, this should possibly deter some colleagues from considering the tetraploid a "good" species. This objection seems rather insignificant, however; the properties of the plant itself must be more important than its mode of origin. This is valid on the condition that the tetraploid does not change back again. If a constant change takes place in both ways, there is no reason for considering the forms different species, as the lines of evolution do not differ.

A more special study of polyploid forms has been made by Peterson (1936) on *Stellaria*. The common weed, *S. media*, seems to be an autotetraploid which has lost two chromosomes ($2n=42$), but forms are known which possess the theoretical number, 44. Two diploid forms are also known, *S. neglecta* and *S. apetala*, which have been treated partly as subspecies or varieties of *S. media*, but which ought to be considered as independent species. Of *S. neglecta* a tetraploid form is known, differing morphologically from *S. media*. This group seems to be very interest-

ing and to throw new light upon the problem of species formation by autoploidy and it ought to induce a certain caution in the treatment of polyploidy in cases where the relationships have not been established by close analysis. The results of Peterson have been disputed by Negodi who has found other chromosome numbers (N. Gior. Bol. Ital. 43).

The part played by polyploidy in species formation was also the subject of an address given by Rosenberg to the 19th Scandinavian Congress of Natural Science, (1936). A recent paper on the same subject by Jensen (1936) has not been available.

For 30 years Heribert Nilsson has been conducting a most interesting work on the genus *Salix*, which is the horror of northern European phytogeographers on account of the extreme variability of the "species" and the overwhelming number of "hybrids" met with in nature. Heribert Nilsson has hybridised different "species" to the most astonishing combinations and has recently published another synthetic plant: *Salix polygena* =

S. [(*purpurea* x *daphnoides*) x (*repens* x *aurita*)]

×

[(*phylicifolia* x *nigricans*) x (*viminalis* x *caprea*)]!

Very interesting is the fact that this plant, of which five specimens have flowered so far, is excellently fertile and breeds true, although *S. phylicifolia* and *S. nigricans* are hexaploids while the others are diploids. Another synthetic species, *S. superlaurina*, is characterised by Heribert Nilsson (1935b) as a "ternary, octoploid allopolyploid of hexaploid and pentaploid parents"!

Contrary to prevailing opinion, Heribert Nilsson has maintained that the number of specific genes within the genus *Salix* must be rather small, but of course the genes must then be very pleiotropic. In the cross *S. viminalis* x *caprea* he has (cp. 1937: 370) assumed five specific genes, as he got back both parents in a progeny of 636 individuals (the theoretical number would be 1 in 1024). One might imagine that the facility with which the *Salix* "species" hybridise might possibly be due to this small number of specific genes. And this might again imply that these "species" are not true species as yet, but that they represent an intermediate stage between ecotypes and species, something between the second and third phases in our "species life history" above.

As a curiosity it might be mentioned that in the case of *S. poly-*

gena the parental forms should, under the assumption of five specific genes, segregate out once in 1.21×10^{24} , i.e., one should need an experimental garden some 10 milliard times the land surface of the earth to cultivate this progeny. This is the reason why *S. polygona* breeds true: the extreme forms are excluded for purely statistical reasons.

If the number of specific genes increases, the possibility of getting back the parents decreases very rapidly. If, therefore, two natural species hybridise and the hybrid is completely fertile and vital, in nature too, not merely in experimental gardens,⁵ the original species will very soon be lost in a rapidly increasing intermediate hybrid population. Such hybrids, however, are hardly to be expected except in crosses between closely related species which have differentiated from a common ancestor in comparatively recent times and have not become genetically stabilised as yet. The genus *Symphytum* represents such a case where the European *S. officinale*, the Persian *S. asperum* and the Caucasian *S. peregrinum* (perfectly "good" species!) hybridise freely when brought together as they are in Europe now. The extreme forms, the "species," are "hybridised away," and the *Symphytum* population of Europe to-day consists to a great extent of intermediate types. Under such circumstances I do not see any reason for keeping the old "species" as species, even if they are still found in a pure state in many places (Fægri 1931).

The above mentioned cross, *Salix viminalis* \times *caprea*, of Heribert Nilsson yielded in F_2 one hypertetraploid plant which is identical with *S. laurina*, a very dubious form that has been known, and in dispute, for more than 100 years (Heribert Nilsson 1928, 1935b).⁶ It has usually been thought to be a hybrid, and highly amusing it is that not less than 12 different species have been supposed to be the parents of this form, and only once has it been in-

⁵This is very important and sometimes overlooked by geneticists. A gene exchange that can take place through a more or less tender and delicate hybrid that must be nursed in experimental plots, is no gene exchange at all in nature and is of no interest whatsoever for the question of natural species delimitation. Experience has shown that even when the hybrid is so vital and fertile as *Geum intermedium* (= *G. rivale* \times *urbanum*), no gene exchange seems to take place in nature; hybrid populations are always rather small and well defined (*cp.* discussion after Clausen 1936).

⁶A Danish author has maintained that *S. laurina* of Heribert Nilsson could not have arisen in this way. The challenge has, however, been demonstrated to be based upon quite false premises (Heribert Nilsson 1935b).

dicated that *S. viminalis* might probably be one of them (by Koch, who also proposed three other parents, none of which was *S. caprea*!).

Another and even more interesting descendant from the same cross, (*S. viminalis* \times *caprea*), is a plant which has been called *S. neo-cinerea* and which cannot be distinguished morphologically from *S. cinerea*, a well known species of southern Scandinavia. *S. cinerea* is tetraploid while *S. neo-cinerea* is at least triploid (Heribert Nilsson 1931b). This plant has not yet been so thoroughly analysed as the synthetic *Galeopsis tetrahit*, and nothing more has been published about it. Prof. Heribert Nilsson has kindly informed me that crosses with *S. cinerea* seem to testify to the identity of the two forms.

Such cases as *Galeopsis tetrahit*, *Salix laurina* and *S. neo-cinerea* demonstrate that even if hybrids occurring in nature are usually intermediate and can be recognised as hybrids by a morphologic analysis, sometimes forms are found that behave like "good" species, the hybrid nature of which cannot be detected by usual taxonomical methods, hardly by the customary genetical analysis; synthesis seems to be the only way to unveil them. The formation of new species in this way is probably too infrequent to be of practical significance in evolution, but theoretically it is very interesting. It is also unknown if the occurrence of such forms is absolutely dependent upon chromosome doubling or other cytologic irregularities, as in the cases mentioned. In those cases where the hybrid behaves like a good species, the problem of its origin is of less importance to taxonomy, but phylogenetically it is very important.

Hagerup has maintained (1932: 19) that as *Empetrum hermafroditum* possesses no new genes as compared with *E. nigrum*, only new combinations of genes, it is hardly to be considered a good species. I can not agree with that; it must be admitted that the morphological difference between the forms is rather insignificant, but it is stable enough to permit even a morphologic distinction; genetically and ecologically the two forms are quite distinct, and even cytologically they are by no means identical, although the difference is quantitative only. As the two forms belong to distinct lines of evolution, we have all reason to treat them as independent species.

The cases of autopolyplody and certain cases of hybridisation, with increase of chromosome number or formation of large intermediate populations, are so far the only cases in which the formation of a new species has been subject to direct observation and analysis.⁷ It is evident, however, that these cannot be the only agents of evolution. The increase of chromosome number is soon stopped by purely numerical limits, and formation of hybrids tends to *reduce* the number of species. These cases certainly represent very important methods of species formation, but, nevertheless, exceptions to the general rule. The development of great and powerful classes of the vegetable kingdom from an inconsiderable beginning can not have taken place in any of these ways. It seems to me that we must consider differentiation due to geographic, or ecologic, isolation followed by selection and genetical stabilisation, as the main agents of evolutionary development.

Finally, I should like to mention an interesting case of natural selection in the experimental field described by Åkerlund (1933). In the field were populations of *Melandrium dioicum*, *M. album* and their hybrid. During winter *M. dioicum*, which is principally a woodland species, suffered much more than *M. album*, which was growing under natural conditions. The hybrids also suffered more than *M. album*, but back-crosses to *M. album* survived better. In a few years this area if left undisturbed should certainly be occupied by *M. album* alone. This little example demonstrates clearly why hybrids between ecologically different forms, species, ecotypes, etc., are rather rare in nature and why ecologically different forms are usually not "hybridised away."

PHYLOGENY OF THE FLOWER OF CONIFERS AND ANGIOSPERMS

Although lying outside the scope of this article, the investigations of Hagerup on this subject should be noted when dealing with phylogenetical problems. Hagerup's first paper (1932) has been mentioned in this journal by Chamberlain (1935). The cardinal point of Hagerup's highly interesting investigations is the idea that the integument of conifers is in reality a *sporophyll* and homologous to the sporophyll in Lycopodinae.⁸ This is supported

⁷ Fagerlin has pointed out (1934) that hybrids between autotetraploids should be completely fertile and constitute new species.

⁸ Personally, I should be inclined to think that the sporophyll of Lycopodinae is *not* homologous with that of Filicinae (and Equisetinae). If the

by a great number of diagrams and drawings showing the earliest stages of development.

In his next papers (1934, 1936) Hagerup analyses the flower of Gnetinae and, employing the same principles, gives exceedingly elegant interpretations of those most difficult structures. He then goes one step further and demonstrates that the same principles even apply to some angiospermous orders, *viz.*, Piperales, Juglandales and Centrospermae (including Cactales). Consequently, according to Hagerup, there seem to be at least two phylogenetic lines leading to the present angiosperms, indicated by the names Lycopodinae – Cordaitinae – Coniferae – Gnetinae – Angiospermae (p.p.) and Filicinae – Cycadinae – Angiospermae (p.p., *e.g.*, Polycarpicae), respectively. That some extinct groups reached the stage of angiospermy independently has been known for some time, but the demonstration of Hagerup, that the present angiosperms belong to different phyla, is very interesting, especially since it presumes a fundamental distinction between the two groups, dating back to the psilophytic stage at least. All complicated structures found in the angiospermous flower should then be of a purely adaptive kind, as they are identical in both phyla. It would also make it probable that *Casuarina* might be a wholly independent type, not related to other angiosperms.

The opinions of Hagerup have been challenged, of course, by some of the great number of investigators working in this field, partly on quite different lines, *e.g.*, Hirmer (1936). Personally, I think that so far as ontogenetic and morphologic investigations can give information about phylogeny, Hagerup has very good arguments for his opinions, and I think his original and thorough work represents an important step toward the definite solution of these problems. The eminent German authority Troll (1934) also supports Hagerup on many points, but strongly rejects the cardinal point, adding that by adhering to that hypothesis, Hagerup only prepares difficulties for his own further explanations. I cannot agree with this, however, for just by adhering to this simple idea,

sporangia of Lycopodinae are developed from multi-sporangial telomes like those of *Drepanophycus* through development of the existing emergences into the *Lycopodium* leaf, while those of Filicinae have developed from uni-sporangial telomes like those of *Psilotophytion* and *Rhynia* through fusion of telomes into the fern, *etc.*, leaf, the difference is certainly fundamental.

Hagerup has, without the aid of any auxiliary hypothesis, solved even the most complicated structures in a very elegant way.

A bibliography will be found in connection with Dr. Fægri's second article, "Some recent publications on phytogeography in Scandinavia," to be published in the next issue.

EXPLANATION OF TERMS

- allopolyploid: a polyploid containing genomes from two or more species.
amphimixis: seed-formation through cross-fertilisation and a normal fecundation process.
amphiploid: a polyploid arising through the addition of the complete chromosome sets of two species.
autecology: the science of the interrelations of the individual plant and its environment.
apomixis: seed-formation without a normal fertilisation and fecundation process.
autogamous: self-fertilising.
autonomous: independent.
autopolyplloid: a polyploid arising through the multiplication of the complete chromosome set of a single species.
autotetraploid: a tetraploid containing $4n$ (not $2n+2n$) chromosomes.
biocoenose: community of plants and animals together.
biotype: a population of individuals with identical genetic constitution.
chorology: the science of distribution of organisms on the surface of the earth (phytogeography and zoogeography *sensu strictu*).
coenose: community.
coenospecies: a population of all those individuals which are capable of gene exchange by direct or indirect crossing (Turesson).
compatible: giving a viable offspring by hybridisation.
continuity of variation: variants forming one group, not divided by \pm empty gaps into more groups.
continuous distribution: the area of distribution not split up by empty gaps where the species &c in question does not occur.
discontinuity: interruption, e.g., in variation curves, leaving the extremes without an intermediate population.
ecospecies: a population of those individuals which give a fertile and viable offspring by hybridisation (Turesson).
ecotype: a population arising through the sorting and controlling effects of the habitat factors upon the heterogeneous species-population (Turesson).
genecology: the doctrine of the ecotype.
genome: the complete (haploid) chromosome set of a species.
hypertetraploid: a tetraploid containing 4 normal genomes and a few extra chromosomes.
mass centrum: the region where a species, genus, &c has its principal occurrence or is best represented.
microspecies: very narrowly delimited species, in contrast to the widely delimited "Linnean" species.
nunatak: rock projecting above and completely surrounded by a glacier.
phylogenetics: the science of the tribal history and descent (phylogeny) of plants and animals.
phytocoenose: plant-community, of any order.
pleiotropic gene: affecting the realisation of a number of external characters.
polymorphism: capability of wide morphological variation.
polyphyly: descent from different ancestors.

polyheity: see polyphyly, *cp.* text p. 409.

provenience: origin, especially the geographical origin, of seeds, plants, &c.
seral units: unstable plant-communities, passing through succession into
other communities, even if external factors like climate or topography
are unchanged.

skerries: low, rocky islands, fringing the Norwegian coast in a broad
border, almost without interruption.

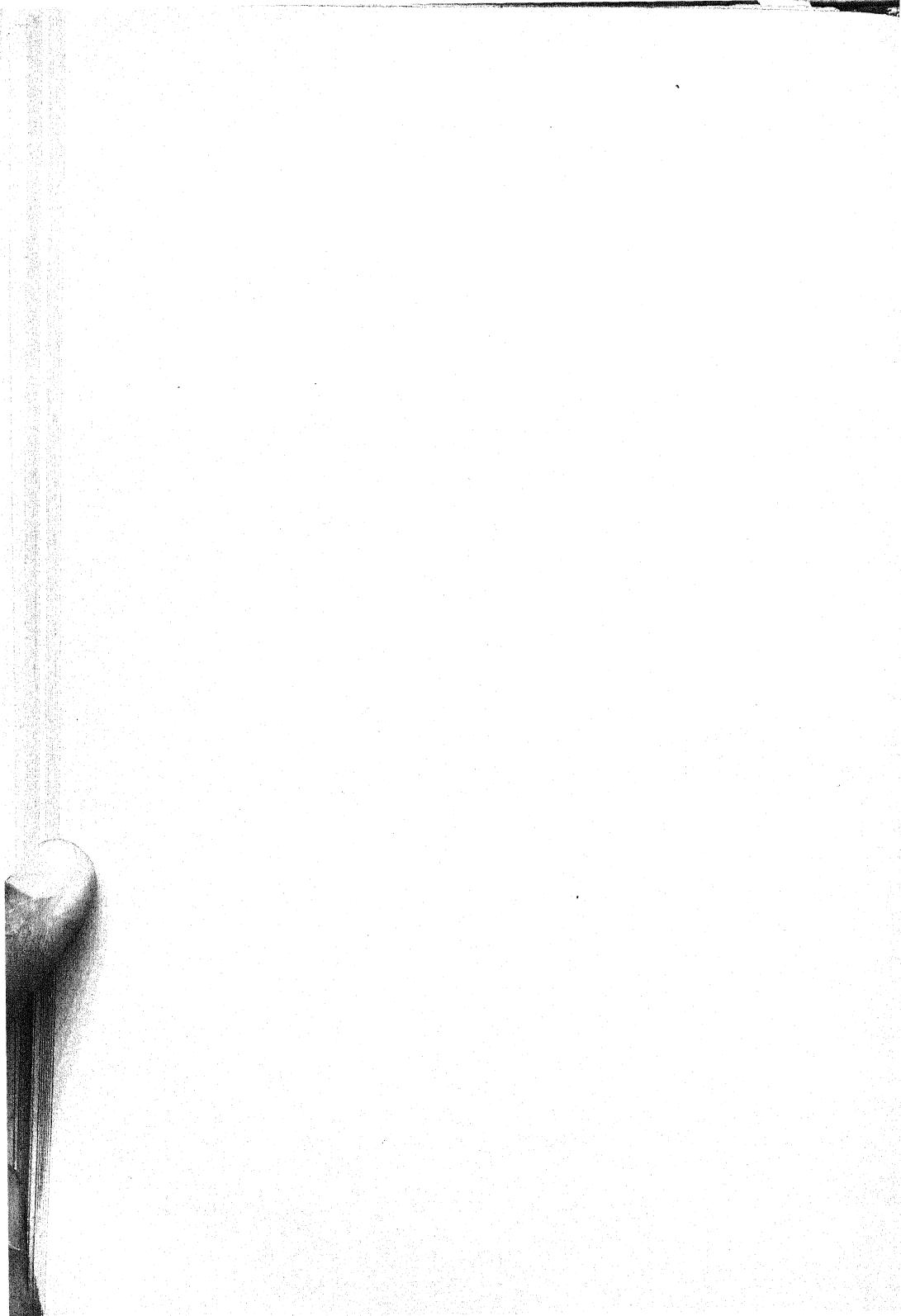
socies: the seral unit corresponding in rank to the sociation among the
stable communities.

synecology: the science of the interrelations of the plant-community and its
environment.

syngameon: intercrossing population.

synusia: the single layers of a plant community, *e.g.*, the tree layer, the
herb layer, &c.

telome: the last, monoaxial branches of a shoot, sterile or fertile (*cp.*
Zimmermann: *Phylogenie der Pflanzen*. 1930, p. 65).



THE BOTANICAL REVIEW

VOL. III

SEPTEMBER, 1937

No. 9

SOME RECENT PUBLICATIONS ON PHYTOGEOGRAPHY IN SCANDINAVIA

KNUT FAEGRI

Bergen, Norway

FLORISTIC PHYTOGEOGRAPHY

Under this heading I shall first mention a paper by Hård av Segerstad (1935), a worthy successor of a series of earlier papers by the same author (1924), Sterner (1922), Almquist (1929) and others on the flora of some larger district of Sweden. It would be of no use to attempt an account of the contents of these volumes. Building upon an old and strong tradition, the Swedish colleagues have been able to collect data, concerning not only botanical, geological and climatological features of the district in question, but also on the whole history of the region, domiciliation, *etc.*, and to form this mass of evidence into a harmonic unity.

The present work (Hård 1935) deals with a very interesting region, the transition from the SW-Swedish oak region to the coniferous forest region further north and at the same time from the sub-oceanic province to the more continental Baltic region. All species occurring here are enumerated, and their distribution within and without the district concerned is viewed in relation to the ecology of the species. Of special interest is the great number of maps of distribution and the references to other cartographical representations.

Two other recent papers, which will also be mentioned quite briefly, are those of Dahl (1934) and Samuelsson (1934). Dahl's paper is the result of ten years' research in the field in Finnmark, that phytogeographically highly interesting northern part of Norway, where a mass of evidence for the mountain flora's surviving the last glaciation in Fennoscandia is found.

Samuelsson has collected data on the aquatic vegetation of Scandinavia over a long period and has now given a very elaborate delineation of the geographical distribution and ecology of these

plants. The paper is certainly of the highest value as a source of reference to everyone who is working with aquatic vegetation.¹

Within a relatively narrow space the Scandinavian peninsula—Norway and Sweden—presents a wider variety of life conditions than perhaps any other area of equal size. From the extreme oceanity of the western coast to a very high degree of continentality in the rain-shadow behind the mountains (in some places even salt enrichment and crystallisation in soil surface is met with during summer), from the middle European oak- and beech-forests to the arctic tundra, from the level of the ocean to the limit of eternal snow and ice, a vast multitude of habitats is found. The plant world of the peninsula, even though relatively poor in species, is highly diversified, therefore, and shows great differences from one place to another, and different "floral elements" have been distinguished long ago.²

Of the floral elements of Scandinavia, two have been the center of interest during the last years, namely, the oceanic ("atlantic") and the alpine. Kötilainen (1933) and Degelius (1935) have given important contributions to the knowledge of the oceanic element, *i.e.*, those species which in Scandinavia show decided preference for the climatically oceanic parts.

Because Scandinavia lies across the most common cyclone paths, barometric depressions on their way eastward are rather sure to reach the peninsula, which has the form of a great dissected table-land increasing slowly in elevation from the Baltic westward and then suddenly dropping down to ocean level. The high mountains on the western coast, which are partly crowned by vast plateau glaciers, act as a very effective barrier to the cyclones, compelling the air currents to ascend and thereby effecting the high precipitation values of western Norway (in places about 3000 mm. in the lowlands, considerably more in the mountains). One important difference from the climatically somewhat similar coast of western North America is caused by the very high temperature of the

¹ *Cb.* Biological Abstracts 10: 17877.

² The term "floral element" has been discussed by Degelius (1935: 10). It has been used in many ways to indicate a common geographic distribution, a common origin, common roads of immigration, *etc.* Degelius proposes to employ the term in its widest sense, leaving to the author to define the special sense in each case. This is very sensible as all attempts to restrict the use to one special sense will certainly prove futile and increase confusion.

Atlantic ocean along the Norwegian coast, giving relatively higher winter temperatures. At the very narrow strip of lowland along the Norwegian west coast there is found, therefore, a number of species which are lacking or scarce in the rest of Scandinavia. Not all of them, however, are really oceanic; many are found rather far eastward further south. Kotilainen has compared the distribution in Scandinavia with that in other parts of Europe to investigate to what extent the "atlantic" species are also really oceanic. It appears that many of them do not at all belong to the oceanic types further south, and their restriction to the oceanic part of Scandinavia is to be explained as an effect of winter temperatures.

Of general interest is the second part of Kotilainen's paper, where the author discusses the causal explanation of the distribution of oceanic species. In some cases the course of the limit of an oceanic species may, at all events within a restricted area, follow an isotherm, e.g., *Ilex* in Norway (Holmboe 1913), but such cases are always exceptions.

It is a curious fact that many of our oceanic species occur at rather high altitudes in the mountains; in such cases it is evident that low temperatures cannot be the factor limiting distribution; humidity must play the most important part. Owing to the form of the country the highest precipitation values are not found in the extreme western skerries, which are rather low, but a little (30–50 km.) eastward, where the mountains rise. The zone of highest thermical oeanity and that of highest hygrical oeanity are separated, therefore, and a number of species distribute themselves very significantly between those zones.

To take into consideration both factors, temperature and humidity, Kotilainen employs an "index of oeanity":

$$I = \frac{P \cdot (a - b)}{10 \cdot (T_1 - T_2)}$$

where P is the average yearly precipitation, T_1 and T_2 the average mean temperatures of the warmest and coldest months, respectively, of the year, and a and b the number of days with a mean temperature either above 0° or 10° C., respectively. According to Gams (1935 b), this formula gives very good results for districts with a sufficient number of days below 0° and above 10° . In arctic ($b = 0$) or subtropic-tropic ($a = b = 365$) climates it is useless.

The hyper-oceanic flora of Scandinavia seems to be confined to those places where I is higher than 150; other more or less decidedly oceanic species go down to about $I = 50$.

Before proceeding to the paper of Degelius, I should like to discuss some others dealing with the problem of the climatic significance of vegetational limits. Enquist (1933) has discussed the relations between timber lines and temperature and has maintained that temperatures themselves, especially not mean temperatures, are not so important as is the duration of a certain temperature. This gives us two unknown values, the duration of the period and the temperature in question. To find these values Enquist has adopted a very original method. He has constructed curves showing the number of days with a certain maximum temperature (in other cases minimum temperatures are employed) for all maximum temperatures within the yearly amplitude. This is done for some places outside and some places inside a certain vegetational limit. It is then found that the curves cross each other in such a way that a small area lies below all curves representing places where the species does occur and above all curves representing places where the species does not occur. The critical value is represented by this area; in the case of the birch in northern Scandinavia the value is at least 26 days with a maximum temperature of 14° or more. Similar calculations give the critical values of other forest trees and Enquist demonstrates how the line representing these values (*e.g.*, 14° and 26 days) cartographically shows a decided coincidence with the area limits of the species in question.

A weak point is that no room is left for humidity. There are certainly vegetational limits which are determined chiefly by humidity as well as limits determined by temperature. In the cases studied by Enquist so far, I should be inclined to think, however, that temperature is everywhere the minimum factor.

The methods of Enquist have been very severely criticised by Langlet (1935) who asserts that his values are based upon too few observations and that no such critical area can be found, as the curves cross each other quite irregularly.

The discussion between Enquist and Langlet is hardly finished as yet, and I shall not enter further upon the question, especially as it seems to be of a secondary nature as compared to the primary problems of the dependence of distributional limits on meteoro-

logical factors. If there is any solution to these problems, ecology must give the key; an intimate knowledge of the aut-ecology of the species in question seems to me a primary condition for any attempt to establish a connection between meteorological data and vegetational limits. But shall we then reach our aim?

The first thing to ascertain, of course, is whether the species inhabits an area as large as possible, or whether for purely historical reasons (including geographic barriers preventing immigration) it is lacking in some part. This is not very easily determined, and it might suffice to refer to the discussion which has been continued for half a century about the western limit of *Picea* in Norway. A classically pure case of historically conditioned distribution areas in animals has recently been given by Ullerot (1936).

Even if the limit is not influenced by historical factors, nor by the activity of man, there are many other problems left. Does this limit depend upon the direct reaction of the plant upon climatic conditions, or do other factors enter? One might imagine, for example, that soil conditions could preclude the occurrence of a species. Such a limit might be climatically determined, but it is evident that the climatological data of the limit have nothing to do with the physiological properties of the species; it is a problem of soil type development. Possibly the soil should also be non-climatically determined. There is a gradual transition from such cases to species of highly specialised habitats which usually show non-climatrical distribution.

The most important group of these are aquatic plants. Water temperatures are much more dependent upon the physical properties of the body of water and of radiation than on the mean temperature of the air (*cp.* Vahl 1906 and Johansen 1906). The remarkable occurrence of a number of aquatic plants among the first immigrants after the glacial age, together with the alpine *Dryas* flora, must be explained in that way, as very few aquatic plants now occur in the alpine region, according to Samuelsson (1934).

But even if all these difficulties are overcome, we are not much nearer our aim. The question arises as to whether temperature or humidity is the deciding factor. In the case of humidity there are the questions as to whether precipitation is necessary or mists suffice, and of the distribution of precipitation throughout the year. With regard to temperature, the relations are still more complicated.

We know that a frost can kill in a few hours and that a certain duration of the vegetation period is essential, but to what extent do plants *need* a frost period? Is frost tolerance in any way influenced by the character of the vegetation period? How shall we express that a heavy snow layer can compensate for low winter temperatures? How is the effect of moderately high temperatures; does one hour of 10° equal one of 24°? And so on. Besides: What are the effects of varying lengths of day at different latitudes, the amount of cloudiness, strength of insolation? All these are fundamental questions which must be considered, and answered too!, before we can attempt to delimit vegetational areas climatically. And these questions cannot be answered by mere reference to a distribution map, be it ever so accurate. It seems to me that a painstaking regional aut-ecologic investigation must be the foundation of climatical interpretation of distribution limits if they are to be more than guesswork.

As pointed out many times (*e.g.*, by Rübel 1935) and forgotten perhaps as often, the complicating factor is the *relativity* of the ecologic demands of the species. One factor may compensate for another, edaphic for climatic, exposure for temperature, *etc.* This modifies and complicates the law of the minimum, the importance of which can hardly be over-rated in phytogeography. The law of the minimum cannot, however, be reduced to a climatologic formula.

The comprehensive study of Gams (1931-1932) demonstrates in how many ways botanists have tried to overcome these difficulties, and with how little success. Even if some vegetational limit might coincide with some climatological line, I do not think we should over-rate its ecologic significance. When Langlet (1936), for instance, finds a pronounced parallelism between contents of dry matter in pine and the number of days with a mean temperature of 6° or more, this hardly means that that value, 6°, is in any way a critical value in the life of the pine. The number of days mentioned is simply an expression of a great complex of factors, some of which we know and some not.

In the same way, the decided parallelism between the limit of *Ilex* in Scandinavia and the January isotherm of 0° (Holmboe 1913) does not mean that this average temperature itself is in any way a critical value in the life of *Ilex*; it must be taken as an ex-

pression of a whole complex of factors, the most important of which most probably is an unknown minimum temperature which within this restricted area is in some way proportional to the mean temperature.

Within a climatically homogeneous region one series of data might rather easily be replaced by another; average temperatures, maximum temperatures, duration values, all of them mean more or less the same, *i.e.*, they represent the same complex of factors. And if one set of values should happen to "fit in" better than others, that does not mean that we have arrived at an ecologically more significant value; it simply means that that special set of values represents a more comprehensive complex of factors.

But while it is comparatively easy to find a set of values which is valid within one climatically homogeneous region, it seems impossible to find any climatical value that can be employed generally, simply because the number of limiting factors is too great; they cannot be included in one single value or formula. In the case of recent vegetation, this is less important because we know the climatic type and know where to look for comparisons, but in the interpretation of fossil quaternary floras it is very important because the climatic type might have changed in the meantime.

Objection has often been raised against the use of ordinary meteorological data on the grounds that they represent quite artificial conditions and that no plant has ever grown under the conditions represented in the ordinary thermometer casings. This is, of course, all true, and for a knowledge of the ecology of the species, or the ecotype, these data are rather useless; quite different values are needed in such cases, soil temperatures, temperatures of different strata of air; in short, micro-climatological measurements. But such data are again rather useless for *regional* comparisons unless regionally investigated—and a regional micro-climatology certainly belongs to a far future.

So we might just as well keep the data of ordinary meteorology which possess the great advantage of referring to known standard conditions all over the world, and we shall consider them as what they really are, namely, expressions of a number of unknown factors. To return to the case of *Ilex* in Scandinavia once more, the coincidence of the limit with the January isotherm of 0° means that in a climate of the western Norwegian type, in places of that

temperature, the favorable characters of the most favorable habitats are just enough to compensate for the adverse character of the general climate; beyond that temperature line, the general climate cannot be compensated for, in western Norway. The absolute values of that compensation process can then be determined only by protracted comparative micro-climatological investigations.

But before we possess those values and the micro-climatological values of all habitats in question, we are, for general use, reduced to employ the values of ordinary meteorology and to supplement them with the diffuse and unsatisfying concept of the climatic type (Klimacharakter, Brockmann-Jerosch) because we cannot provide the exact numbers.

Nevertheless, the climatic type as indicated by the complete course of temperature curves may form the base of a comparison of localities and proveniences with regard to artificial afforestation, as demonstrated by Hagem (1931) in his comparative studies of the climates of western Norway and western North America.

Even if I thus feel obliged to take up a rather negative attitude toward the attempts to define vegetational limits climatically, I can not agree with Turessons concept of the value of the plant species as a climatic indicator (1932 b). If a species has a given range of distribution and this distribution is natural and complete, we have represented within this range all those climatic conditions under which the species in question is able to live. Under such circumstances it is rather immaterial whether the species consists of one or more ecotypes; its total range is the same. Another thing is that ecotypes, if constant!, naturally are much *finer* climatic indicators, if not too restricted edaphically.

In an impressive volume (1935), Degelius has dealt with the non-crustulose oceanic lichens of Scandinavia. Twenty-two species are discussed most thoroughly and a very detailed account is given of their history of discovery in Scandinavia, distribution within and outside Scandinavia, habitat, vertical distribution, mode of dispersal and variability.³ Of great interest are the chapters II, V and VI, dealing with the general problem of the oceanic element. The different area types are compared with areas belonging to the eu-oceanic and sub-oceanic elements as defined by Troll, and a great many sub-elements are distinguished. With regard to distribution

³ C. Biological Abstracts 10: 17840.

within Scandinavia, a great number of distribution maps, chiefly of mosses and most of them original, is of great value.

Discussing the causes of oceanic distribution, Degelius finds that temperature certainly plays a part, especially in the case of eu-oceanic species and, indirectly, through its effects on humidity. Nevertheless, humidity is usually the deciding factor, especially precipitation. Fogs do not play a great part generally, even though they may be of importance locally and even more so further south (*cp.* the great importance ascribed to fogs as a source of water in the Nambimb desert by Walter 1936). The habitats are also characterised by very favorable humidity conditions; usually two or more species are found together, showing their common demands.

It is very interesting to note the great stress which Degelius lays upon humidity, almost to the exclusion of temperature conditions, as causing the distribution of oceanic species. Holmboe (1924-1925: 8), on the other hand, maintains that temperature is the deciding factor, as in our broken terrain some place of sufficient humidity will always be found. Kotilainen (1933) takes an intermediate view. It seems that this controversy is mainly due to the different groups of plants taken into consideration. Holmboe deals chiefly with vascular plants, Degelius with lichens and mosses, and Kotilainen with vascular plants and mosses. For the vascular plants, which have a rather effective water transport system and which are usually more or less protected against excessive evaporation by means of cuticle, *etc.*, humidity must naturally play a minor part. On the other hand, for the lower cryptogams, which have no such system of transport but which are more or less dependent upon the water that is absorbed through the whole surface and which have no real means for checking evaporation, humidity must tend to be the minimum factor in determining distribution.

Nordhagen (1933, 1935, 1936 b), Holmboe (1936, 1937), Nannfeldt (1935 b) and others have recently dealt with the Scandinavian mountain flora. Contrary to the former interpretation of this flora as having immigrated from the south and east after the glacial age, a great number of species are now supposed to have "wintered" during the last glaciation at ice-free refuges on the Norwegian coast. Vaguely formed long ago, this theory has been strengthened by each successive author.

The first paper of Nordhagen (1933) gives a wide outlook on

the conditions for human habitation in northern Europe during the glacial age with special reference to the very interesting finds of apparently paleolithic implements in Finnmark. Later, Bøe (1936) concluded from archeological evidence that they probably represent an immigration from the southeast, along the Russian and Lapponian rivers, reaching the coast of Norway after the last glaciation.

This archeological question is of minor importance in this connection, however, but it has served to stimulate interest about the problem of survival of the Scandinavian flora during the glacial age. A great many of our alpine plants are quite trivial and rather evenly distributed all over the area where the mountains are high enough, e.g., *Salix herbacea* or *Loiseleuria procumbens*. Most of these species are acidiphilous, but more demanding species are found, e.g., *Dryas octopetala* or *Thalictrum alpinum*. These also occur wherever the soil possesses the right qualities. There is, however, another group, our "rare" mountain plants, which are restricted to one or both of two areas, one in central Norway and the other in northern Scandinavia, from the arctic circle northwards. Many of these species show remarkable distributional disjunctions, and it seems obvious that they cannot have immigrated after the glacial age; they must have wintered in refuges somewhere on the Scandinavian peninsula.

Nordhagen has tried to localise these refuges, to find not only the regions but the exact spot where the wintering took place, in the same way as has been done by Fernald in North America. Especially in southern Norway many winterers are not now found within the refuges; they have migrated eastward since the ice melted away. The principal reason for this migration, especially for the total disappearance in the one-time refuges of southern Norway, is to be found in the great oceanity of the western Norwegian climate. In the usually acid humus soil and closed plant communities of this climate our rare and demanding alpine plants cannot compete, many of them having but small competitive power, being confined to loose gravels and other open communities.

By utilising the evidence given by all our previously known alpine plants, and even more by the evidence given by forms insufficiently known as yet, the taxonomy of which Nordhagen has cleared up himself, he has been able to give a much more exact picture of the wintering process than we have had. The new forms referred to

are, first, the perennial *Papaver* species of Scandinavia (Nordhagen 1931) which have proved very interesting, for there are represented not less than five different species and a number of forms of lower taxonomic rank. Some of these forms are very particular with regard to occurrence, being found in only one locality and giving, therefore, excellent evidence of the site and individuality of the refuges. Other very interesting forms have been found in the genus *Arenaria*, a "new" species of which, *A. humifusa* Wahlgren (1812) (= *A. cylindrocarpa* Fernald 1914), was recently rediscovered by Nordhagen (1935).

By means of the evidence collected in this way, and guided by the occurrence of glacial erosion-forms, nunataks, etc., Nordhagen has now (1935, 1936 b) very clearly indicated where to look for glacial refuges in Scandinavia. There must have been a great number of clearly distinct and separate refuges all along the northern coast of Norway (possibly also further eastward), the most southerly lying a little south of the arctic circle. Then there has been a gap—at all events, we have no botanical evidence for the occurrence of refuges—until we reach a latitude of 62–63°, where important refuges must have existed, containing the remarkably rich alpine flora of the central mountains. Further south, the location of refuges is less certain, even though there are good reasons to suppose that they existed there also.⁴

The somewhat curious distribution of refuges with a maximum in the extreme north is explained by the form of the coast line. Where the latter is convex, as in the two principal refuge districts, the ice streams coming from the interior are allowed to spread over a wide area and are broken up, consequently, into a series of separate lobes. In the middle region, where the coast line is concave, the ice streams are forced together to form one mighty glacier that reaches far out (Holmboe 1937).

The rare mountain plants are of great importance because of their limited power of spreading, being still found in well defined and restricted areas which show decided relations to the refuges. But there is, of course, no reason why only these rare and demanding species should have survived the glaciation; they were certainly

⁴ Very interesting parallels to the occurrence of refuges in Norway are presented by the recent demonstration of ice-free refuges in Iceland (Lindroth 1931) and even in eastern Greenland (several authors, cf. Gelting 1934, 1936).

accompanied by more trivial species, but the latter have been able to spread all over the mountains. It is evident, therefore, that *all* our alpine plants *might* be glacial winterers, but this *possibility* alone does not preclude the possibility that some of them might also have immigrated from the south after glaciation. We know that tundra plants, remains of which are found along the ice border in middle Europe, followed the retreating ice through Denmark to Scania, where remains of species like *Dryas octopetala*, *Salix herbacea*, etc., are found in many places. However, after the Salpausselkä stage in Finland—Suomi and its equivalents in Sweden and Norway the climate had ameliorated so much that no tundra was formed at the ice front, pine and birch forests invading the area immediately. It is not impossible, therefore, that no alpine plants really managed to migrate from the tundras of middle Europe to the Scandinavian mountains by following the retreating ice front and that the whole alpine flora of Scandinavia survived the last glaciation at the coast refuges.

As it is, there are some alpine species which most probably immigrated from the south; they are not found in the mountains, however, but in the southern lowlands. The most outstanding example is the middle European alpine *Euphrasia salisburgensis*, which in Scandinavia is found in Gothland only, while the closely related *E. lapponica* replaces this species in the Scandinavian mountains. The occurrence of a number of common alpine plants in the lowlands, also in southern Sweden (Lid and Zachau, 1928), might be due to secondary invasion from the mountains, but possibly also to immigration from the south. Turesson has demonstrated (1927) that some of these lowland types represent special ecotypes, clearly different from the alpine ones. He maintains that the alpine ecotypes immigrated immediately after recession of the ice sheet and reached the mountains, while the lowland ecotypes did not immigrate until the climate had attained a suitable character. This theory, which is a direct consequence of Turesson's view on the absolute stability of the genetical constitution of the ecotype, seems rather unnecessary. If the lowland forms have immigrated from the middle European tundra, while the mountain forms came from the refuges of the Norwegian coast, the genetical difference is easily understood; and even if they have the same origin, both coming from the south or west, the post-glacial period has certainly been

long enough to permit differentiation of ecotypes. In one of his earliest papers on the subject, Turesson (1922 b: 350) seems to favour the idea that some ecotypes have come into existence quite recently. This hypothesis would account much better for such cases as the lowland ecotypes of alpine species, too.

Holmboe (1936) maintains that *Nigritella nigra* has also survived glaciation in both groups of refuges on the Norwegian coast, for its distribution is now typically bi-centric in the same way as that of many other winterers. This is very interesting because *Nigritella* is an essentially sub-alpine plant which nowadays rarely occurs in the alpine region. One might ask how great a part of our sub-alpine flora has also been able to survive the last glaciation in the refuges. This represents quite a new and different side of the problem, and the question has been formulated so recently that very little has been done in answer to it. I shall, therefore, not enter upon it here. There are many sub-alpine species with remarkable distribution, which it should be very tempting to explain in this way, e.g., *Gentiana purpurea*, *Campanula barbata*, *Phyteuma spicatum* which occur in Norway only (*Phyteuma* also in Denmark), but not in Sweden.

Later on (1937), Holmboe suggested that *Hippophaë rhamnoides*, *Arabis petraea* and other sub-alpine plants have also wintered on the Norwegian coast. He has also established a very interesting route of migration of the sub-alpine winterers from the Norwegian coast through the totally glaciated Trondheim district, across the low mountain passes and into middle Sweden. It is the same route that was later taken by a number of immigrant species which in this way reached middle Sweden by way of the Norwegian valleys or the Norwegian coast.

As well as alpine and sub-alpine plants, arctic and sub-arctic sea-shore species might also have survived in the refuges. *Polygonum Raji * norvegicum*, which is almost endemic to the refuge districts (Samuelsson 1931), obviously represents one such case, and others are possibly to be explained in the same way.

But even if the western Norwegian refuges might possibly have contained not only alpine but also sub-alpine species, I think it is extremely difficult to accept the idea that also the "atlantic," i.e., the oceanic element of the Scandinavian flora, survived the glacial age in the refuges, as advanced long ago by Stejneger (1907) and

more recently by Lindroth (1931), even if some facts seem to speak for this hypothesis. In a late-glacial gyttja deposit I found evidence that the first vegetation of the extreme southwestern part of the Norwegian coast after the recession of the ice was an extreme snow patch (chionophilous) vegetation, like that which is now found at 1200 m. altitude in southern Norway. After that there followed a long and pronounced dwarf-birch stage (Faegri 1935 b). One can hardly imagine *Digitalis* or *Ilex*—nor the red deer, *Cervus elaphus L.*⁵—to have lived under such conditions. The oceanic element must have survived glaciation somewhere else, e.g., in the now disappeared North Sea continent, or west of Ireland, as suggested by Degelius (1935), and must have immigrated to the Norwegian coast afterwards, when climatic conditions were more suitable.⁶

Because of the great disjunctions, the same reasoning can not be applied to the alpine species. We cannot suppose a species to have migrated thousands of kilometers after the last glaciation without leaving a trace behind. The little group of about 20 species which are called west-arctic and which occur also in Greenland and North America but not in the western parts of Siberia nor further south in Europe, is very significant in this respect. I shall return to them later.

Nannfeldt (1934, 1935 a, 1935 b) has started to clear up the extremely difficult genus *Poa* and has achieved some very interesting results. What was formerly called *P. laxa* does not belong to the genuine *P. laxa* of the Alps, but to a distinct, closely related species, *P. flexuosa* Smith seq. Nannfeldt (not to be confused with the great number of other *P. flexuosa*s!), which represent the *P.*

⁵ The existence of a separate subsp. *atlanticus* Lönnberg in western Norway has been disproved by Ingebrigtsen (1922-23).

⁶ In a recent paper, Roi (1936) seems to doubt that all biogeographical elements peculiar to Ireland have survived the glacial epoch in the country; he is inclined to favour the hypothesis of later immigration in a number of cases. Unfortunately, the treatment is not quite complete, as the lichens, which are very important in this respect and which have been discussed very thoroughly by Degelius in the same paper (1935), are omitted. Even if I agree with Roi that each individual species must be treated separately, I cannot see how the distribution of species such as those constituting the very disjunct Mediterranean and Lusitanian and even more the Hiberno-American elements can be explained if we do not presume that the great majority of them wintered in the country itself, or if this is impossible, in the continent which must have existed west of Ireland during the glaciations. In connection with the Hiberno-American element the small number of American rhizopods ought be remembered which in Europe have their only occurrence in Ireland and western Great Britain (Wailes in Cash and Wailes 1915).

laxa group in Scandinavia, Scotland and Iceland, while other species represent the group in the Alps, etc. (*P. laxa*, *P. minor*), the Transylvanian Alps (*P. Nyaradiana*) and North America (*P. Fernaldiana*).

The distribution of *P. flexuosa* is very interesting as it is coincident with that of *Arenaria * norvegica*, which is also endemic to the shores of the northern Atlantic ocean. I should like to touch here upon the questions of endemism and species formation, which have also been discussed by Nordhagen (1935) and Nannfeldt (1935 b). Those species which occur also in middle Europe or western Siberia may, of course, have had a more continuous distribution during some interglacial period and may have immigrated to Scandinavia at any point of time. If we agree that they survived the last glaciation in Scandinavia, we must suppose that they did not immigrate later than the transition from the great ice age to the last interglacial period. The case of the extremely disjunct west-arctic species is more difficult. These species can not be supposed to have crossed the present north Atlantic ocean. Only two possibilities seem to be left: either the north Atlantic ocean did not exist during the last interglacial period—the theories of continental bridges or continental flow might account for that—or the west-arctic floral element represents an even older flora which survived even the great ice age in Scandinavia. So far, it seems a matter of taste as to which of these hypotheses shall be considered the more probable.

Although our alpine flora has thus been isolated since the beginning of the last interglacial period (at least), we find very few endemic species other than such genera as *Taraxacum* and *Hieracium*, upon which I shall not enter. Some of the *Papaver* species of Nordhagen are certainly endemic, even if their distribution outside Scandinavia is not properly known. Then there are some species which are closely related to but different from corresponding species in middle Europe, as in the case of *Euphrasia lapponica*—*E. salisburgensis* mentioned above. A list is given by Nannfeldt (1935 b: 66). I do not think any of these has been genetically tested against the others. It seems very probable that in future other cases will be found, the populations of middle Europe and Scandinavia being genetically different. On the whole, however, such cases are strikingly few and the differences surprisingly small.

Considering that the isolation of these populations has lasted some 50,000 to 100,000 years (*cp.* Gams 1935 *a*), one might expect that there had been ample time for the establishment of more profound specific differences than those found.

The case of the *Poa laxa* group is very interesting in this connection, as the original population has been split up in different parts of the world, middle Europe, Scandinavia, America. Each of these partial populations has differentiated into a new species, and this must have taken place before the last interglacial period. The present distribution of *P. flexuosa* must have been established before the last glaciation, *i.e.*, during the last interglacial period. When the last glaciation began, *P. flexuosa* must have been present in Great Britain, Iceland and Scandinavia, and have found refuges wherein to survive the last glaciation in all these districts. But during all the time that the British, Icelandic and Scandinavian populations have been separated, no perceptible differentiation seems to have taken place.⁷

The *Poa laxa* group is thus taxonomically very conservative; although the different populations have been separated during two glaciations at least, species differentiation has not gone further than to the establishment of those very closely related species described by Nannfeldt. This is rather remarkable as most species within the genus *Poa* possess all characteristics of being "young": great ranges of variation, diffuse limits, great tendency toward hybridisation.

Papaver seems to represent quite a different case; the forms described by Nordhagen are for the most part fairly distinct although we have all reason to suppose that at least some of the subspecies are comparatively young, having differentiated during the last glaciation. The stability of genetical constitution within this genus seems considerably lower than within *Poa*. As it is, many of the differences met with in the genus *Papaver* are such as might be supposed to be localized in a single gene. If so, the higher rate of development is possibly explained in that way. In grasses, all differences are more inconspicuous, of course, and it is not quite justified, therefore, to make direct comparison.

⁷ Turesson has challenged the papers of Nannfeldt and Nordhagen rather scornfully (1936), reproaching them for the great number of hypotheses. Even though it might be admitted that genetical investigations of the forms described might have given more certain information about the different new species, I cannot agree with the criticisms.

But even in the case of *Papaver*, which must be considered one of relatively rapid differentiation, 50,000 years have just been sufficient to give us some very closely related species or subspecies. It seems rather improbable that geneticists shall ever be able to demonstrate the origin of new species through differentiation in their experimental gardens. One can easily understand then how geneticists may be induced to take such a negative position toward the whole problem of species formation as did Heribert Nilsson in his inaugural address at the University of Lund (1935 a).

THE PRESENT STATE OF PHYTOSOCIOLOGY

Phytosociology has a long tradition in Scandinavia, the names of Norrlin, Hult, Kihlman, Warming and Raunkiær belonging among the classical names in this branch of botanical science. The greatest influence has been exercised, however, by Sernander, not only as an investigator, but even more as a teacher at the University of Uppsala. He is the father of what is now known as the Uppsala school of phytosociology (not to be confused with the Uppsala school of quaternary geology of some decennia ago, which was also led by Sernander). The Uppsala school has been dominating in Scandinavian phytosociology for two decennia. Characteristic of the first of these decennia were the theoretical discussions; a great number of papers, rather contentious in many cases, was indited, turning partly against other Scandinavian investigators, but as often against foreign, especially those of middle Europe, represented by the Zürich-Montpellier school (Rübel, Braun-Blanquet, Pavillard). The results of these heavy theoretical polemics were evident about 1925. The differences of opinion within the school being rather insignificant, the Uppsala school was at that time a rather well established unit which took a decidedly controversial position against the opinions advanced by middle European investigators (with some exceptions, e.g., Gams, who joined the Uppsala school at a very early point of time (Gams 1918; cp. Du Rietz 1930 b).

Reviewing now the great number of theoretical polemic papers of this first decennium, one could perhaps be inclined to think that they were purely speculative, having little or no connection with the study of real vegetation. This was not the case, however, for as early as 1920, Du Rietz, Fries, Osvald and Tengwall in their joint paper state that they dispose of a material of no less than about

20,000 vegetational analyses, and the number has since been increasing. Much of this material has been published in a series of impressive monographs, from the earlier ones of Fries (1913), Smith (1920) and Tengwall (1920-25), still based mainly upon the "classical" concepts, to the later ones of Osvald (1923), Nordhagen (1927), Booberg (1930), Thunmark (1931) and Lindquist (1931). But even more is still unpublished and will perhaps remain so, owing to the enormous costs of publishing such material. Especially is it to be regretted that the studies of Du Rietz on the Island of Jungfrun, which are, in fact, fundamental to the whole Uppsala school, have been published only very incompletely, in the form of an excursion guide (1925) and partly also in other publications.

A milestone in the development of phytosociology is formed by Du Rietz's paper of 1930 (*b*) which at the same time embodies the theoretical basis of the Uppsala school and tries to establish a connection with the Zürich-Montpellier school. During the time which has passed since then, the connections between those two very active centers of phytosociological research have gradually gained strength and importance. Even if there are fundamental differences of opinion which will possibly remain forever, a decided adjustment has taken place, facilitating collaboration.

The fundamental unit of phytosociology is defined thus by the Uppsala school (Du Rietz 1930 *b*: 307): "Eine Soziation ist eine stabile Phytocoenose von wesentlich homogener Zusammensetzung, d.h. wenigstens mit konstanten Dominanten in jeder Schicht." The corresponding definition of the Zürich-Montpellier school runs (Braun-Blanquet and Pavillard 1922: 7): "L'unité sociologique fondamentale est *l'Association*. Chaque association se caractérise principalement par ses *espèces caractéristiques*." The profound difference of those two definitions and of the corresponding phytosociological units induced Du Rietz to drop the former term "association" of the Uppsala school and adopt the new term "sociation" originally proposed by Rübel for a unit of a lower rank than the association, in order to point out the existing differences and also to prevent the confusion likely to arise when the same term is employed in different ways. But the adoption of the new term did not, and does not, mean that Du Rietz, or anybody else, has dropped his former association concept and accepted the view of Pavillard on the association (in the sense of the Zürich-Montpellier school)

as "the fundamental unit of phytosociology." As Du Rietz has pointed out (1935: 584), there are many units, and which of these is to be considered the fundamental one, and which name is to be attached to that unit, is a matter of secondary importance. The change proposed by Du Rietz in 1930 was a *purely terminological* one, as expressly stated by Du Rietz (1930 b: 304).

When Pavillard (1935), as well as Ashby (1936), earlier in this journal have claimed that the method of the Uppsala school has been "abandoned by its inventors" (Ashby p. 224) or that "of this Uppsala method . . . nothing now remains" (Pavillard p. 214), it is thus absolutely contrary to the facts.⁸

The period of approximation and collaboration between the two phytosociological schools mentioned reached a provisional culmination in the "conclusion of peace" at the Sixth International Botanical Congress, where a resolution was passed by the geobotanical section on the proposal of Braun-Blanquet, Du Rietz and Nord-

⁸ Ashby even goes so far as to refer to the Uppsala school as "a failure" (p. 225), which can hardly be said to be a fair statement of other opinions. In the paper of Ashby, which otherwise is much more objective than that of Pavillard, I should like to point out that the terms of common language: "moor," "meadow," "heath," etc., do not designate sociological units, and few things have been so hampering to phytosociological research as the endless discussions about the sociological meanings of these terms and their delimitation. Nordhagen has demonstrated (1936 a) that the only way of solving the question is to discard these terms altogether. I quite agree with Ashby that a personal subjective delimitation of communities (not "choice of constants," nobody is able to realize all possible constants of a community at a glance!) must always be the primary and can never be replaced by any mechanical or statistical method, and I do not think any phytosociologist of the Uppsala school will disagree with Ashby on that point; but the statistical analysis is necessary to show if our subjective opinion is correct or not. It is exactly the same procedure which is employed in taxonomy: at first one gets an impression of the delimitation of forms, and then one studies the characteristics to see if that impression holds true. The renowned "law of Raunkiær" serves exactly the same purpose: if the frequencies do not arrange themselves according to that law—which is purely statistical and not due to any particular property of the plant-community, as was for some time believed by certain phytosociologists of different schools—it is a sign that something must be wrong with the community in question. With regard to sampling at random, Ashby seems to misunderstand one point. If one is going to make a study of the *Parnassia* population of a meadow, one must, of course, take care that only plants of *Parnassia* are collected and not also of *Ranunculus*, etc. In the same way, if one is going to make a study of a special community, one must take care that samples are taken from that community alone. In many cases, e.g., on sea shores, one might certainly apply—and does apply—methods like those suggested by Ashby, but in many other cases, where ecologic conditions vary greatly within short distances, one has to choose more carefully before taking samples.

hagen, accepting both the sociation and the association as defined by the respective schools and recommending the alliance as the common higher unit (Proceedings, p. 402).

As the sociation usually is a much smaller unit than the association, one should expect that the sociations described by Scandinavian investigators could be united into associations. This has, however, not been practised as yet; on the contrary, it seems to be very difficult to find clearly established associations in the Scandinavian vegetation. This is undoubtedly a major reason why the controversies between Scandinavian and Swiss phytosociologists have arisen. The Swiss vegetation is formed by a great number of species, many of which are sociologically very significant, while the Scandinavian vegetation is formed by a much smaller number, most of which have a rather wide sociological amplitude. With the possible exception of a small number of very rare species occurring mainly in one or a few associations, the Scandinavian phytosociologists have searched in vain for character species upon which to found their associations. On the other hand, even the most trivial communities, containing nothing but the commonest species, show through the variation of their quantitative composition a remarkable faculty of reacting upon and indicating the ecologic qualities of the habitat. The sociation is, in fact, a finer instrument of ecologic research than the association. The Zürich-Montpellier phytosociologists also admit that "es gibt auch Assoziationen bei denen man unsonst nach Treuen sucht" and that "in Gesellschaften ozeanischen Klimas kommt . . . auch die Treue nur abgeschwächt oder gar nicht zur Geltung" (Rübel, 1933).

At this point I should like to make an excursion with regard to the relationships between sociology and syn-ecology. The sociologic units are the units of syn-ecology in the same way as taxonomic units are also the units of aut-ecology. Accordingly, sociology is the basis of syn-ecology. One should be very careful, therefore, not to delimit sociological units, plant communities, by ecologic characters, as the result will be a *circulus vitiosus* of no value whatsoever. But if sociological units are to be of value in syn-ecological research, those units must be employed which permit the most penetrating analysis of syn-ecological relationships of a given vegetation, i.e., the sociation, at all events in northern Europe.

To recapitulate the present state: The leading Uppsala and

Zürich-Montpellier phytosociologists base their researches upon two different fundamental units, each recognizing the value of the unit employed by the other school.⁹ That they have not been able to agree upon the same unit, is to a great extent because of practical considerations. In the rich and varied flora of middle Europe the association is a handy instrument of research, giving a general view of the ecologic relationships without losing itself in less important particulars; in the poorer vegetation of northern Europe the sociation is that instrument of research by the aid of which one can penetrate deeply into the ecology even of a floristically very monotonous and poor district.

When not only Pavillard but also Ashby (1936: 224) is troubled about the Scandinavian vegetation, which is "pulverized into an innumerable multitude of minute rudimentary groups . . . generally covering a very restricted area," it remains to be remarked that the first to suffer from the drawbacks of this procedure should have been the Scandinavian phytosociologists themselves, but as yet no lament has arisen from them on this point. On the contrary, the last paper of Nordhagen (1936 *a, cp.* below) demonstrates that not only can these units be handled very easily and grouped together in larger classes, but also that they are of wide distribution. On the other hand, it might be (even if it is far from certain!) that in the richer flora of middle Europe a division of vegetation into sociations should result in, if not "an innumerable multitude," at all events an unwieldy number of sociations; but so far, no such experiences have been made.

⁹ With the exception of Pavillard, who voted against the said resolution at the Amsterdam congress (Cain, 1936, *cp.* also Pavillard, 1935, and Du Rietz, 1936). It must be regretted that Pavillard has used the pages of this journal for continuing a polemic which represents now a past stage in the development of phytosociology and for deepening differences of opinion which everybody else seems now to be intent upon removing. The paper of Pavillard contains a number of statements which are not only subjective, but direct misstatements. To disprove or comment upon them lies outside the scope of the present article, but as an example I just want to point out that far from giving up "the attempt to secure official consecration of his minor floristic (!) units" (Pavillard, 214), Du Rietz invited the Sixth International Botanical Congress to adopt the sociation. And the congress did so—against two votes, one of which was Pavillard's! Very remarkable also is the statement that "the present purely empirical method of determining sociations justifies us in considering them as absolutely incapable of filling the rôle of fundamental units." How shall a vegetational unit be determined if not empirically? At all events, none of the Scandinavian phytosociologists is so gifted that he can sit down at his writing-table and determine the fundamental units by intuition.

It is characteristic to note that the opposers always inform us about the 164 sociations described by Osvald (1923) from "a stretch of moorland five miles by eight" (Ashby, p. 224), but they forget to mention that by analysing vegetation so closely, Osvald discovered the three fundamental complexes of raised bog development, the regeneration, stagnation and erosion complexes, which dominate the bogs at different periods and a knowledge of which gives an explanation to the whole peat bog development and the key to post-glacial climate (*cp.* Granlund, 1932). In the same way Sernander long ago discovered the regeneration process by applying the same narrow unit (v. Post and Sernander, 1910), even if its theoretical foundation was at that time not developed.

"Glücklicherweise lassen sich dennoch die nordischen und mitteleuropäischen Einheiten sozusagen auf dieselbe Wellenlänge transformieren und zwar mittels der von Braun-Blanquet aufgestellten. . . . Begriffe Gesellschaftsverband (Alliance) und Gesellschaftsordnung (Ordre)" (Nordhagen, 1936 *a*: 4). Even though the search for character species upon which to found associations in Scandinavia has been rather futile, Nordhagen has demonstrated that there are to be found species which we can consider character species of the larger units mentioned. As it is, their number is small, according to the relative poverty of our flora; nevertheless, starting with the sociations described by himself and other investigators of the Uppsala school, he has succeeded in establishing a division of the sub-alpine-alpine vegetation of Norway on quite middle European principles, and he has even demonstrated that most of the orders and many of the alliances are common to the mountains of middle Europe and Scandinavia.

While this development has been in progress in Sweden and to a smaller extent also in Finland-Suomi¹⁰ and Norway, Danish phytosociology has been remarkably little influenced. This is partly due to the nature of the country; while the other Scandinavian countries have a low density of population and possess large areas of natural, or semi-natural, vegetation, Denmark is densely populated and above all one of the best cultivated countries of Europe;

¹⁰ Owing to the nature of the country, a great deal of the vegetational research has here had more practical aims, chiefly considering the economically all-dominant forestry, and has operated with more practical vegetational units, forest types, moor types, etc. Under the eminent leadership of Cajander, this school has achieved results of great practical value; but I shall not enter further upon that matter here.

consequently, there is not much natural vegetation left. Besides, most of the natural vegetation of Denmark belongs to a few types, sea shores, lakes and heaths. The problems of phytosociology have been rather different, therefore, from those of the other Scandinavian countries.¹¹ The dominating influence of Raunkiær has also been instrumental in bringing about the special development of Danish phytosociology. The interests have been directed against the individual plant and its ecology more than against the community, its sociology and ecology.

An excellent representative of this trend in Danish phytosociology is the paper published recently by Iversen (1936). Very important is the author's differentiation between hydrotypes, *i.e.*, *morphological* adaptations to the nature of the habitat, classified by the usual terms of xerophytes, mesophytes, *etc.*, and hygrobe types, referring to *ecologic* relations, designated as xerobes, hygrobes, *etc.* Such differentiation ought to be adopted as soon as possible, for a deplorable confusion now rules on this point. To prevent misunderstanding, Iversen also employs terms like halobes instead of halophytes to designate ecologic relations to the salt concentration of the soil. Pointing out the insurmountable difficulties in determining these ecological factors exactly, he proposes relative scales only,—a very sound plan.

Very important also is his "diagram of ecologic affinities," a method of determining objectively the ecologic affinities between species. Even if it is rather simple, the method is difficult to explain without the diagram at hand, so I shall have to refer the reader to the original work, which also contains applications of methods and concepts in analysis of vegetation.

The Raunkiær method of vegetation analysis has great practical and scientific advantages, and as a method for determining frequencies it has the great advantage of being almost independent of the aspect of the vegetation, as the species is identified with the location of its hibernating organs. Frequency is, however, but one of the many questions of phytosociological research, and the original Raunkiær method does not give anything more. Bøcher (1935), who has further developed the method to an excellent instrument for determining constancy, distance of shoots and homogeneity,

¹¹ The investigations of Danish and Norwegian botanists in Greenland during the last years have mainly been floristic. A paper on vegetation has been published by Bøcher (1935).

therefore proposes that it may be combined with some method of determining the covering of vegetation, either the more discretionary method of Hult-Sernander usually employed by the Uppsala school or the more objective New Zealand point method, which in Scandinavia has been employed by Lindquist (1931).

In a lecture presented to the Nineteenth Scandinavian Congress of Science Sørensen proposed some new concepts and terms of phytosociology. As I did not listen to the lecture and the short abstract given in the Proceedings (Sørensen, 1936) is not very elucidative, I shall not enter upon these concepts. I hope Sørensen will return to them in the paper on Greenland vegetation upon which he is now working.

During recent years Du Rietz has repeatedly advocated the advantages of employing synusiae as a base for phytosociological research. This opinion was maintained long ago by Gams; Lippmaa has since been working on similar lines in Estonia, and quite recently Cain (1936) has recommended their use in phytosociological field work in North America. For more complete information I must refer to Du Rietz (1936) and Cain. Lippmaa, who used to call his fundamental synusial unit "association unistrate" and consequently voted against the said resolution at the Amsterdam congress (together with Pavillard (Cain: 671), but on different premises), later accepted the terminology of Du Rietz and Gams (1936: 583), according to which the synusiae corresponding to sociation, association and alliance are to be called society, union and federation, respectively. As yet we have not seen much of how the synusia method will work when applied to the actual problems of vegetational research, even though some Scandinavian investigators have also employed it in their work (Du Rietz, 1932, Tunmark, 1931, Lindquist, 1931). I think it is well worth trying and that there is great possibility that it will facilitate the description and delimitation of societies as well.

It seems that the Zürich-Montpellier phytosociologists are not very much interested in the study of synusiae as yet. Pavillard (1935: 214) writes about Du Rietz's "long series of synusiae, which interest scarcely anyone," and Braun-Blanquet (1936: 28) denies their value as autonomous units. The reasons for this attitude are not quite obvious. One might suppose that the use of a relatively comprehensive fundamental unit might obscure the relationships of

the smaller one-layer units, but this appears hardly probable, as the original association concept of Lippmaa seems to correspond in rank to that of the Zürich-Montpellier school.

At all events, it is known that a small number of societies, synusiae, may combine in different ways into a number of sociations, as demonstrated by the authors quoted above and also by Gams (1936) from the Austrian Alps and by Osvald (1935) from North American bogs. It seems that the interdependence of the layers varies a great deal. Thunmark (1931) states that the synusiae of aquatic vegetation are practically independent of each other and combine in all ways. In other cases one might presume a certain dependence between the synusiae. The question about interdependence or autonomy of synusiae should be taken up for thorough investigation before deciding for or against their use in phytosociological research.

At all events, it is obvious that our present method, analysing the composition of the three or four most prominent layers, does not and cannot give a full account of the composition of the bio-coenose, not even of the phytocoenose, as a number of synusiae, in the soil, on boulders, on tree trunks, *etc.*, are omitted or very incompletely dealt with.

While actual successions in peat bogs, lakes, *etc.*, have been very thoroughly studied by a number of Scandinavian investigators, the general successional problems, the climax problems, have not interested Scandinavian phytosociologists very much during the later period. The phytosociologic concepts are primarily static in contrast to the purely or primarily dynamic concepts of some schools (*cp.* Carpenter, 1936) which have also influenced the Zürich-Montpellier school. Those Scandinavian investigators who have dealt with the climax concept, mostly decline accepting the mono-climax idea (*cp.* Du Rietz, 1930 *b*).

Studying the vegetation on "new" soil formed by the recession of glaciers in western Norway (1933), I found it very difficult to deal with more complicated successions without a climax concept. I then developed my view on succession in the following way: Succession is a matter of competition. If the vegetation in a certain habitat is composed of those species which under circumstances in force have the highest competitive power—and it must be remembered that the plants themselves are a very important part of the

habitat—no succession can take place. If we look apart from those successions which are brought about by the introduction of species, formerly not represented in the region, the main motive of succession is in a stable climate soil development. Under the influence of vegetation, the soil develops toward one climatically conditioned type, the soil climax. Corresponding to this soil climax we have a vegetational climax, namely, a community of plants (whatever may be its sociologic rank) which have the highest competitive powers under the circumstances given.

The first to be observed in this connection is the duration of the process. On a crag of granite, polished by the ice-age glaciers, where no soil has been formed during thousands of years, one might easily predict that the soil climax would not establish itself for perhaps millions of years. The lichen community which dominates the rock now will do so for ages to come, and it seems a little misapplied to call such a stage a pioneer stage, even though I admit that these somewhat incongruous consequences alone are not reason for condemning the whole concept.

Another thing seems more serious: The soil climax doctrine was developed in a flat region where the rock is covered with mighty layers of highly decomposed and weathered materials. This is the case, however, only where topographical relief is quite mature; most of the earth has, I think, a rather immature relief, and great parts have also been glaciated quite recently; the loose masses are consequently relatively fresh. A general realization of the soil climax, and of the vegetational climax, seems, therefore, to be postponed until relief differences are levelled and the peneplane stage reached. This is a matter of an immensely far future, separated from us by millions of years, if it is ever to be realised; and what is even more important, in a levelled world, climatic conditions will certainly be radically different from those of present-day western Norway. So the whole mono-climax idea miscarries on account of its imminent measurelessness. We shall never be able to determine its real nature and must leave it out of our discussions on actual vegetation problems.

Du Rietz has given a modified climax concept in his treatment of vegetation regions (1930 b: 344). Within such regions there is usually one phytocoenose of rather high rank which is dominating; sometimes there might be a few mutually substituting phytoco-

noses. Such a *dominating coenose*, the Hauptzönose of Schmid (1922), is practically, but theoretically not, identical with the climax of the monoclimax school. The important difference is that while all other communities are, according to the mono-climax concept, stages in the development toward this one community only, there are, according to the idea of the dominating phytocoenose, a number of other communities which are of such great stability and duration that they serve as landmarks in the successional development and which probably never develop further, at all events, not unless climatical changes occur. Besides, there is a number of clearly transitory communities. The other stable communities are then considered simply what they are: independent communities of great importance and distribution beside the dominating one, not as stages in a development which will possibly never take place. While the dominating community, or communities, serves to characterise the region, is an exponent of the whole region, the other stable communities must be considered as having equal rights in sociology. The principal difference between this view, which is, I think, shared by most Scandinavian phytosociologists of today, and the view of the mono-climax school, is that to the latter an overwhelming number of the communities met with in nature are *seral units (sociies)* and a few *climax units (sociations)*; the former consider the great majority of communities stable (*sociations*) and reserve the term *seral units* for those which are clearly transitory.

ACKNOWLEDGMENTS

During the last two years I have had the opportunity to meet almost all those Scandinavian colleagues whose works I have dealt with in my two articles, and I have discussed with them various points of their work. I want to thank all of them for the pleasant hours I have spent with them, and especially my thanks are due Professors Nordhagen (Bergen) and Du Rietz (Uppsala) for advice in connection with the preparation of these articles.

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VEGETATIVE REPRODUCTION OF THE FERN SPOROPHYTE

ILDA McVEIGH

University of Missouri

INTRODUCTION

During the latter half of the nineteenth century proliferation ("regeneration") in ferns was studied by many botanists. A beginning was made of tracing the histological origin of the new parts in a few species. Much of this work, however, was obscurely published, and little attention has been given it by recent workers in the general field of regeneration. The present review assembles and organizes this scattered literature.

Proliferation from the sporophyte only is included here. The chief interest of students of regeneration is to trace the relationship between the new parts and the tissues, differentiated and otherwise, of the parent. Since the gametophyte consists almost entirely of parenchyma, such questions scarcely arise.

Because the term regeneration has been variously defined by different authors, its use is avoided here. The early investigators use it to refer to the ability of organisms to replace lost parts. Many zoologists give it the same meaning. Botanists commonly use it to mean the development of an entire new organism from a detached part of an adult or embryo. According to some authors, the term is restricted to the formation of new parts exactly like the parts lost in position, number and form. Some include apospory and apogamy. Some restrict regeneration to the formation of parts from differentiated cells while others include the development of buds and preformed rudiments. The term proliferation is used in a broader sense to include all of these and in the discussion which follows refers to the production of any type of growth from the vegetative tissues of a plant, other than the roots, stems and leaves normally formed. This discussion is restricted almost entirely to vegetative reproduction which is the term used for the formation of an entire new individual.

Proliferation may occur from root, stem or leaf. Sometimes it occurs normally in the development of the plant; in others, only as the result of an artificial stimulus. Sometimes the proliferated

structure is gametophytic and sometimes sporophytic. Occasionally both types of tissues are produced from the same plant. In some ferns only new plant parts are produced while in others entire new individuals are developed.

LIST OF FERNS WHICH REPRODUCE VEGETATIVELY

Because of the number of species which reproduce vegetatively and the amount of literature dealing with proliferation in ferns, it is convenient to list the species and references in tabular form. In the following tables I have included only those species which give rise to new plants vegetatively. In many the origin of the new plant has not been histologically investigated and only brief mention of the mode of reproduction was made in the works cited. The ferns are grouped in four tables: (1) those in which the new plants are formed from leaves or stolons; (2) those in which new plants are formed from roots; (3) those which reproduce aposporously; (4) those in which the new individual develops only after an artificial stimulus. Whenever it is known, the place of origin of the new plant has been indicated. The names of species, genera and families are, as far as possible, those in Christensen's Index Filicum. Where synonyms are used, the first is the binomial now accepted and the second is that used by the author or authors cited. It is sometimes impossible, to be sure, what species an author means, especially when he has failed to include the authority of the binomial. For example, Hofmeister (1857) wrote about *Asplenium Belangeri* but failed to indicate whether it was *A. Belangeri* Bory = *A. longissimum* Bl. or *A. Belangeri* Kze. = *A. tenerum* Forst.

In the discussion which follows, the accepted binomials are used, the names used by the investigators, if not the same, being placed in parentheses.

From Leaves and Stolons

Gleicheniaceae

I. *Gleichenia* Sm. Buds in angles of forkings of frond. Kerner & Oliver (1895).

1. *G. cryptocarpa* Hk. Hooker & Baker (1868).
2. *G. Cunninghamii* Hew. Hooker & Baker (1868).
3. *G. flabellata* R. Br. Hooker & Baker (1868).
4. *G. flagellaris* (Bory) Spr. Hooker & Baker (1868).
5. *G. hirta* Bl. Hooker & Baker (1868).

6. *G. pedalis* (Klf.) Spr. Hooker & Baker (1868).
7. *G. revoluta* Hk. & Bak. Hooker & Baker (1868).
8. *G. tenera* R. Br. Hooker & Baker (1868).
9. *G. umbraculifera* (Kze.) Moore. Hooker & Baker (1868).
10. *G. vestita* Bl. Hooker & Baker (1868).

Hymenophyllaceae

I. *Trichomanes* L.

1. *T. botryoides* Klf. Buds lateral, on elongated rhachis. Hooker & Baker (1868), Kupper (1906).
2. *T. diffusum* Bl. Buds in axils of pinnules. Kupper (1906).
3. *T. diversifrons* (Bory) Mett.—*T. dimorphum* Mett. Buds lateral on elongated rhachis. Engler & Prantl (1902), Kupper (1906).
4. *T. elegans* Rich. Buds lateral on elongated rhachis. Kupper (1906).
5. *T. pinnatum* Hedw. Buds lateral on elongated rhachis. Goebel (1930), Kupper (1906).
6. *T. proliferum* Bl. Buds in axils of pinnules. Kupper (1906).
7. *T. pyxidiferum*. Atkinson (1894), Bower (1887).

Marattiaceae

I. *Angiopteris* Hoffm.

1. *A. evecta* (Forst.) Hoffm. Buds from stipules or leaf cushions. Goebel (1930), Raciborski (1902), Sachs (1882).

II. *Marattia* Sw.

1. *M. alata*. Buds on isolated scales or stipules at base of leaves. Druery (1896), Hofmeister (1857).

Osmundaceae

I. *Osmunda* L.

1. *O. regalis* L. Buds on rhachis. Birkenhead (1899).

Parkeriaceae

I. *Ceratopteris* A. Brongn.

- C. thalictroides* (L.) Brongn.—*C. cornuta* le Prieur. Buds on lamina. Bally (1909), Beyerle (1932), Engler & Prantl (1902), Goebel (1887), (1930), Haberlandt (1914), Holm (1925), Holscher and Lingelsheim (1915),

Howe (1931), Jenman (1885), Kerner and Oliver (1895), Poirault (1893), Sachs (1882), Schenk (1881).

Polypodiaceae

I. *Adiantum* L.

1. *A. calcareum* Gardn. Bud at tip of elongated rhachis. Hooker & Baker (1868), Kupper (1906).
2. *A. capillus-junonis* Rupr.—*A. cantoniense* Hance. Bud at tip of elongated rhachis. Hooker & Baker (1868), Kupper (1906).
3. *A. capillus-veneris* L. Bulbils in place of sori. Druery (1895).
4. *A. caudatum* L. Bud at tip of elongated rhachis. Goebel (1902), Hooker & Baker (1868), Kupper (1906).
5. *A. deflectens* Mart.—*A. dolabriforme* Hk. Bud at tip of elongated rhachis. Goebel (1902), Kupper (1906).
6. *A. delicatulum* Mart. Bud at tip of elongated rhachis. Kupper (1906).
7. *A. Edgeworthii* Hk. Bud at tip of elongated rhachis. Goebel (1902), (1905), (1930), Hooker & Baker (1868), Kerner & Oliver (1895), Kupper (1906).
8. *A. lunulatum* Burm. Bud at tip of elongated rhachis. Hooker & Baker (1868), Kupper (1906).
9. *A. Mettenii* Kuhn. Buds on pinnules. Poirault (1893).
10. *A. pumilum* Sw. Bud at tip of elongated rhachis. Kupper (1906).
11. *A. rhizophorum* Sw. Bud at tip of elongated rhachis. Kupper (1906).
12. *A. rhizophyllum* Schrad. Bud at tip of elongated rhachis. Kupper (1906).
13. *A. Schweinfurthii* Kuhn. Bud at tip of elongated rhachis. Kupper (1906).
14. *A. soboliferum* Wall. Bud at tip of elongated rhachis. Kupper (1906).

II. *Anogramma* Link

1. *A. schizophylla* (Bak.) Diels—*Gymnogramma schizophylla* Bak. Jenman (1885).

III. *Aspidium* Sw.

1. *A. cameroonianum*. Buds on rhachis in axils of pinnules (also on midribs of pinnules). Kupper (1906).
2. *A. effusum* Sw. Buds on rhachis usually near apex. Kupper (1906).
3. *A. ilicifolium* Fée. Buds at tips of fronds. Jenman (1885).
4. *A. Krugii* Kuhn. Bud at tip of elongated rhachis. Kupper (1906).
5. *A. macrophyllum* Sw. Jenman (1885).
6. *A. rhizophyllum* Pr. Bud at tip of elongated rhachis. Kupper (1906).

IV. *Asplenium* L.

1. *A. abscissum* Willd.—*A. firmum* Kze. Bud at apex of frond. Hooker & Baker (1868).
2. *A. achilleifolium* (Lam.) C. Chr.—*A. pro-longatum* Hk.—*A. rutaefolium* Kze. Bud at tip of elongated rhachis. Goebel (1902), Kupper (1906), Poirault (1893).
3. *A. alatum* H. B. Bud at tip of elongated rhachis. Kupper (1906).
4. *A. amboinense* Willd.—*A. fejeense* Brack. Bud near apex of rhachis. Hooker & Baker (1868), Kupper (1906).
5. *A. anisophyllum* Kze. Buds on rhachis usually near apex. Kupper (1906).
6. *A. attenuatum* R. Br. Bud at apex of leaf. Hooker & Baker (1868).
7. *A. auriculatum* (Thbg.) Kuhn—*A. Thunbergii* Kze. Bud on rhachis usually near apex. Kupper (1906).
8. *A. Belangeri*. Buds on rhachis in axils of pinnules (or on the bases of the pinnules). Atkinson (1894), Engler & Prantl (1902), Heinricher (1878), (1894), Hofmeister

- (1857), Kupper (1906), Palisa (1900), Sachs (1882), Schenk (1881), Zimmerman (1881).
9. *A. bianthemum* Gilbert. Bud at tip of leaf. Gilbert (1897).
 10. *A. bifidum* Pr.—*A. lineatum* Sw. Buds on the lamina. Kupper (1906).
 11. *A. bulbiferum* Forst. Buds on the lamina (usually near vein). Atkinson (1894), Bower (1923), Dobbie (1921), Druery (1896), Engler & Prantl (1902), Haberlandt (1914), Heinricher (1878), (1881), Hooker & Baker (1868), Kerner & Oliver (1895), Kupper (1906), Palisa (1900), Zimmerman (1881).
 12. *A. caudatum* Forst. Buds on rhachis in axils of pinnules (also on lamina). Kupper (1906).
 13. *A. commutatum* Mett. Buds on rhachis, usually near apex. Kupper (1906).
 14. *A. compressum* Sw. Buds on rhachis, usually near apex. Kupper (1906).
 15. *A. depaupertum* Fée. Bud at tip of elongated rhachis. Kupper (1906).
 16. *A. dimorphum* Kze. Buds on the lamina. Kupper (1906).
 17. *A. Dregeanum* Kze. Buds on rhachis usually near apex. Kupper (1906), Hooker & Baker (1868), Zimmerman (1881).
 18. *A. ebeneum proliferum*. Bud from rhachis. Waters (1903).
 19. *A. ebenoides* Scott. Bud at tip of frond. Gray (1908), Scott (1875), Wherry & Trudell (1930).
 20. *A. emarginatum*. Pal. Beauv. Buds on rhachis usually near apex and also in sinus at tip of pinnae. Hooker & Baker (1868), Kupper (1906).
 21. *A. enatum* Brack. Buds on lamina. Kupper (1906).

22. *A. exiguum* Bedd. Buds at tips of fronds and sometimes also at tip of pinnae. Hope (1899).
23. *A. extensum* Fée. Buds on rhachis. Hooker & Baker (1868).
24. *A. Feei* Kze. Buds on rhachis, usually near apex. Kupper (1906).
25. *A. Finlaysonianum* Hk. Buds on rhachis usually near apex. Kupper (1906).
26. *A. flabellifolium* Cav. Bud at tip of elongated rhachis. Dobbie (1921), Hooker & Baker (1868), Kupper (1906).
27. *A. flabellulatum* Kze. Bud at tip of elongated rhachis. Kupper (1906), Zimmerman (1881).
28. *A. fontanum* (L.) Bernh.—*A. refractum* Moore. Druery (1896).
29. *A. fragile* Pr. Buds on rhachis. Hooker & Baker (1868).
30. *A. furcatum*. Buds on lamina. Goebel (1887), Sachs (1882).
31. *A. galipanense* Hier. Bud at tip of elongated rhachis. Kupper (1906).
32. *A. Gautieri* Hk. Buds on rhachis usually near tip. Kupper (1906).
33. *A. gemmiferum* Schrad. Buds on rhachis, usually near tip. Hooker & Baker (1868), Kupper (1906).
34. *A. Gibertianum* Hk. Bud at tip of elongated rhachis. Kupper (1906).
35. *A. Glenniei* Bak. Bud in sinus at apex of pinnae. Davenport (1888).
36. *A. Hallii* Hk. Bud at tip of elongated rhachis. Kupper (1906).
37. *A. integrifolium* Prl. Buds on the base of the lamina. Kupper (1906).
38. *A. Karstenianum* Kl. Bud at tip of elongated rhachis. Kupper (1906).
39. *A. Krausii* Moore. Buds on stolons. Kupper (1906).

40. *A. laciniatum* Don. Bud at tip of elongated rhachis. Kupper (1906).
41. *A. Lauterbachii* Christ. Buds on stolons. Goebel (1930), Kupper (1906).
42. *A. lineatum* Sw. Buds on lamina. Kupper (1906).
43. *A. longicauda* Hk. Buds on rhachis near apex and also at tip of pinnae. Kupper (1906).
44. *A. longissimum* Bl. Buds lateral on elongated rhachis. Kupper (1906).
45. *A. lunulatum* Sw.—*A. erectum* Bory. Buds on rhachis, usually near apex. Engler & Prantl (1902), Hooker & Baker (1868), Kupper (1906).
46. *A. macrophyllum* Sw. Buds on rhachis usually near apex. Kupper (1906).
47. *A. Mannii* Hk. Buds on stolons. Goebel (1902), (1930), Kupper (1906).
48. *A. monanthes* L.—*A. monanthemum* (Murr.) L. Bud on rhachis in axils of pinnae. Jemman (1885), Kupper (1906).
49. *A. myriophyllum* (Sw.) Pr.—*A. rhizophyllum* Kze. Bud at tip of elongated rhachis. Kupper (1906).
50. *A. normale* Don—*A. multijugum* Wall. Buds on rhachis near apex or in axils of pinnae. Kupper (1906).
51. *A. oceanicum* C. Chr.—*A. obtusilobum* Hk. Buds on stolons. Goebel (1902), (1930), Kupper (1906).
52. *A. paleaceum* R. Br. Bud at tip of elongated rhachis. Hooker & Baker (1868), Kupper (1906).
53. *A. Palmeri* Maxon. Bud at apex of elongated rhachis. Maxon (1909).
54. *A. paradoxum* Bl. Buds on rhachis usually near apex. Kupper (1906).
55. *A. partitum* (Kl.) C. Chr.—*A. radicans* Sw. Bud at tip of elongated rhachis. Kupper (1906).

56. *A. pellucidum* Lam.—*A. hirtum* Kaulf. Buds in axes of pinnae. Kupper (1906).
57. *A. persicifolium* J. Sm. Buds on rhachis usually near apex. Kupper (1906).
58. *A. pinnatifidum* Nutt. Bud at apex of frond. Eaton (1881), Gray (1908).
59. *A. plantagineum* Sw. Buds on base of lamina. Kupper (1906).
60. *A. Poolii* Bak. Buds on rhachis near apex and also at the tips of pinnae. Kupper (1906).
61. *A. projectum* Kze. Buds on rhachis in axes of pinnae. Kupper (1906).
62. *A. quitense* Hk. Buds on stolons. Kupper (1906).
63. *A. radicans* L.—*A. rhizophorum* L. Bud at tip of elongated rhachis. Hooker & Baker (1868), Jenman (1885), Kupper (1906).
64. *A. regulare* Sw. Buds on rhachis usually near apex. Kupper (1906).
65. *A. resectum* Br. Buds on rhachis usually near apex. Kupper (1906).
66. *A. rhachirhizon* Raddi. Bud at tip of elongated rhachis. Kupper (1906).
67. *A. rutaceum* (Willd.) Mett. Bud at tip of rhachis. Hooker & Baker (1868), Kupper (1906).
68. *A. ruta-muraria*. Druery (1896).
69. *A. Sandersoni* Hk. Bud at tip of elongated rhachis. Kupper (1906).
70. *A. sherburgense* Bak. Bud at apex of leaf. Baker (1890).
71. *A. tenerum* Forest. Buds on rhachis in axes of pinnae. Kupper (1906).
72. *A. triphyllum* Pr. Buds on rhachis. Hooker & Baker (1868).
73. *A. vagans* Bak. Bud at tip of elongated rhachis. Hooker & Baker (1868).
74. *A. vittaeforme* Cav. Buds on rhachis usually near apex. Kupper (1906).

75. *A. viviparum* (L.f.) Pr. Buds on lamina. Atkinson (1894), Bower (1923), Goebel (1902), Haberlandt (1914), Heinricher (1878), (1894), Hooker & Baker (1868), Kupper (1906), Schenk (1881).
76. *A. vulcanicum* Bl. Buds on rhachis usually near apex. Hooker & Baker (1868), Kupper (1906).
77. *A. Zenkerianum* Kze. Buds on rhachis usually near apex. Kupper (1906).

V. *Athyrium* Roth.

1. *A. Filix-femina* (L.) Roth—*A. Filix-femina* Bernh. Buds on upper surface in axils of pinnae and on lower surface in place of sori. Atkinson (1894), Druery (1885), (1896), Sachs (1882).
2. *A. nigripes* (Bl.) Moore—*A. Clarkei* Atkins Buds on rhachis, usually near apex. Kupper (1906).

VI. *Blechnum* L.

1. *B. asperum* (Kl.) Sturm—*Lomaria aspera* Kl. Bud at tip of elongated rhachis. Hooker & Baker (1868).
2. *B. auriculatum* Cav.—*B. hasatum* Kl. Buds on stolons. Atkinson (1894).
3. *B. lanceola* Sw. Buds on stolons. Goebel (1930).
4. *B. sprucei* C. Chr.—*L. caudata* Bak. Bud at the of elongated rhachis. Hooker & Baker (1868).

VII. *Camptosorus* Link

1. *C. rhizophyllum* (L.) Link—*Scolopendrium rhizophyllum* Endl. Bud at tip of elongated rhachis. Atkinson (1894), Campbell (1928), Druery (1896), Goebel (1902), Gray (1908), Hooker & Baker (1868), Kupper (1906), McVeigh (1934), Poirault (1893), Tweedy (1880), Yarbrough (1936).
2. *C. sibiricus* Rupr.—*Scolopendrium sibiricum*

Hk. Bud at tip of elongated rhachis. Kupper (1906).

VIII. *Cheilanthes* Sw.

1. *C. Belangeri* (Bory) C. Chr.—*C. varians* Hk.—
Pellaea Cambodiana Bak. Bulbils in place of sori. Beddome (1910), Goebel (1930).

IX. *Cystopteris* Bernh.

1. *C. bulbifera* (L.) Bernh. Buds on rhachis in axils of pinnae. Atkinson (1894), Bower (1923), Campbell (1928), Clute (1909), Druery (1896), Engler & Prantl (1902), Heinricher (1896), Holm (1925), Hooker & Baker (1868), Jenman (1885), Kunze (1849), Kupper (1906), Matouschek (1894), Palisa (1900), Rostowzew (1894).

X. *Dennstaedtia* Bernh.

1. *D. rubiginosa* (Klf.) Moore. Buds on upper surface of lamina. Bower (1923).

XI. *Diplazium* Sw.

1. *D. bantamense* Bl.—*Asplenium bantamense* Bk. Buds on rhachis usually near apex. Kupper (1906).
2. *D. celtidifolium* Kze.—*A. celtidifolium* Mett. Buds in axils of pinnules and also on under side of end pinnules. Atkinson (1894), Bower (1923), Goebel (1902), Heinricher (1878), Holm (1925), Kupper (1906), Palisa (1900).
3. *D. proliferum* (Lam.) Thouars—*Anisogonium decussatum* Pr.—*Asplenium decussatum* Sw. Buds in axils of pinnules. Atkinson (1894), Goebel (1887), Hooker & Baker (1868), Kupper (1906), Sachs (1882).
4. *D. setosum* Pr.—*Asplenium setosum* Pr. Bud at tip of elongated rhachis. Kupper (1906).
5. *D. Virchowii* (Kuhn) Diels—*Asplenium Virchowii* Kuhn. Buds on base of lamina. Kupper (1906).

XII. *Doryopteris* J. Sm.

1. *D. pedata* (L.) Féé—*Pteris pedata* L. Buds on base of lamina. Kupper (1906).

XIII. *Dryopteris* Adans.

1. *D. asplenoides* (Sw.) O. Ktze.—*Polypodium asplenoides* Sw. Buds at tip of leaf. Jenman (1885).
2. *D. erythrosora* (Eat.) O. Ktze.—*Aspidium erythrosorum* Eat. Atkinson (1894).
3. *D. Filix-mas* (L.) Schott—*Aspidium Filix-mas* Atkinson (1894), Engler & Prantl (1902), Goebel (1887), Hofmeister (1857), Sachs (1882), Schenk (1881).
4. *D. oreopteris* (Ehrh.) Maxon—*Lastrea montana* Moore. Druery (1896), (1903).
5. *D. prolifera*—*Lastrea prolifera*. Bulbils in place of sori. Druery (1896).
6. *D. pusilla* (Mett.) O. Ktze.—*Nephrodium pusilla* Bak. Buds in axils of upper pinnae. Hooker & Baker (1868).
7. *D. refracta* O. Ktze.—*Nephrodium deflexum* J. Sm. Buds on rhachis usually near apex. Kupper (1906).
8. *D. reptans* (Gmel.) C. Chr.—*Polypodium reptans* Sw. Buds on elongated rhachis. Hooker & Baker (1868), Jenman (1885), Kupper (1906).
9. *D. reticulata* (L.) Urban—*Meniscium reticulatum* (L.) Sw. Buds on mid-veins of lower pinnae. Eaton (1906).
10. *D. spinulosa* (Mull.) O. Ktze.—*Aspidium spinulosum* Sw. Buds on petiole. Engler & Prantl (1902), Hofmeister (1857).

XIV. *Fadyenia* Mk.

1. *F. Fadyenii* (Mett.) C. Chr.—*F. prolifera* Hk. Bud at tip of elongated rhachis. Engler & Prantl (1902), Hooker & Baker (1868), Kupper (1906), Poirault (1893).

XV. *Hemionitis* L.

1. *H. arifolia* (Burm.) Moore—*H. cordata* Roxby. Buds on base of lamina. Kupper (1906).

2. *H. palmata* L. Buds in sinuses of lamina.
Beyerle (1932), Kupper (1906), Jenman (1885).

XVI. *Leptochilus* Kl.

1. *Leptochilus auriculatus* (Lam.) C. Chr.—*Acrostichum punctulatum* Sw. Buds on rhachis usually near apex. Kupper (1906).
2. *L. cuspidatus* (Pr.) C. Chr.—*Acrostichum repandum* Bl. Bud at tip of leaf. Hooker & Baker (1868).
3. *L. gaboonense* (Hk.) C. Chr.—*Acrostichum gaboonense* Hk. Buds on rhachis usually near apex. Hooker & Baker (1868), Kupper (1906).
4. *L. heteroclitus* (Pr.) C. Chr.—*Acrostichum flagelliferum* Wall.—*Chrysodium flagelliferum* Mett. Buds on lamina and on the elongated rhachis. Engler & Prantl (1902), Goebel (1887), Hooker & Baker (1868), Kupper (1906), Sachs (1882).
5. *L. Linnaeanus* Fée—*Acrostichum Linneanum* Hk. Buds on rhachis usually near apex. Hooker & Baker (1868), Kupper (1906).
6. *L. subcrenatus* (Hk. & Grev.) C. Chr.—*Acrostichum proliferum* Hk. Hooker & Baker (1868).
7. *L. virens* (Wall) C. Chr.—*Acrostichum semicordatum* Bak.—*Acrostichum virens* Wall. Buds at tip of rhachis and also at end of lateral pinnae. Hooker & Baker (1868), Kupper (1906).

XVII. *Matteuccia* Todaro

1. *M. Struthiopteris* (L.) Todaro—*Onoclea Struthiopteris* Hoffm.—*Struthiopteris germanica* Willd. Buds on stolons. Atkinson (1894), Clute (1909), Druery (1896), Engler & Prantl (1902), Goebel (1887), Hofmeister (1857), Sachs (1882).

XVIII. *Nephrolepis* Schott

1. *N. cordifolia* (L.) Pr.—*N. Pluma* Moore—*N. tuberosa* Pr.—*N. undulata* J. Sm. Stolon and tubers. Engler & Prantl (1902), Goebel (1887), (1930), Heinricher (1907), Hofmeister (1857), Jenman (1885), Kunze (1849), Sachs (1882), Schenk (1881).
2. *N. hirsutula* (Forst.) Pr. Stolons & tubers. Goebel (1930), Heinricher (1907).

XIX. *Phyllitis* Ludwig

1. *P. hybrida* (Milde.) C. Chr. Buds on leaves. Bornmiller (1916).
2. *P. Scolopendrium* (L.) Newm.—*Scolopendrium officinale* Lam. Buds on surface of leaf. Druery (1896), (1901).

XX. *Polybotryta* Humb. & Bompl.

1. *P. appendiculata* (Willd.) J. Sm.—*Acrostichum appendiculatum* Willd.—*Acrostichum Hamiltonianum* Wall. Buds on rhachis near apex. Hooker & Baker (1868), Kupper (1906).

XXI. *Polypodium* L.

1. *P. proliferum* Pr. Buds on the elongated rhachis. Hooker & Baker (1868), Kupper (1906).
2. *P. vulgare* L. Buds in place of sporangia. Druery (1895).

XXII. *Polystichum* Roth.

1. *P. aculeatum* (L.) Schott—*Aspidium aculeatum* Sw.—*Aspidium proliferum* R. Br.—*Aspidium subinerme* Kze.—*P. angulare* Pr.—*P. platyphyllum* (Willd.) Pr. Buds on rhachis usually near apex. Dobbie (1921). Druery (1896). Engler & Prantl (1902). Hooker & Baker (1868), Kupper (1906), Maxon (1909), Newman (1854), Sim (1879).
2. *P. auriculatum* (L.) Pr.—*Aspidium auriculatum* Sw. Buds on rhachis, usually near apex. Kupper (1906).

3. *P. christiana*e (Jenm.) Und. & Maxon. Bud at tip of leaf. Maxon (1909).
4. *P. craspedosorum* (Maxim.) Diels. Bud at tip of elongated rhachis. Engler & Prantl (1902), Kupper (1906).
5. *P. decoratum* Maxon. Bud at tip of elongated rhachis. Maxon (1909).
6. *P. dissimulans* Maxon. Bud at tip of elongated rhachis. Maxon (1909).
7. *P. Harrisii* Maxon. Bud at the tip of leaf. Maxon (1909).
8. *P. Hookerianum* (Pr.) C. Chr.—*Aspidium caducum* Wall. Buds on rhachis in axils of pinnules or on the bases of the pinnules. Kupper (1906).
9. *P. lepidocaulon* (Hk.) J. Sm. Bud at tip of elongated rhachis. Kupper (1906).
10. *P. Maximowiczii* (Bak.) Diels. Bud at tip of elongated rhachis. Engler & Prantl (1902), Kupper (1906).
11. *P. Plaschnickianum* (Kze.) Moore—*Aspidium Plaschnickianum* Kze. Bud at tip of elongated rhachis. Engler & Prantl (1902), Hooker & Baker (1868), Jenman (1885), Kupper (1906).
12. *P. polystichiformis* (Fée) Maxon. Bud on rhachis below apex. Maxon (1909).
13. *P. rhizophorum* (Jenm.) Maxon. Bud at tip of leaf. Maxon (1909).
14. *P. trapezoides* Sw. Bud at tip of elongated rhachis. Kupper (1906).
15. *P. triangulum* (L.) Fée—*P. ilicifolium* Fée. Bud at tip of elongated rhachis. Engler & Prantl (1902), Kupper (1906), Maxon (1909).
16. *P. Underwoodii* Maxon. Bud at tip of leaf. Maxon (1909).
17. *P. vestitum* (Forst.) Pr.—*Aspidium vestitum* Sw. Holm (1925).

18. *P. viviparum* Féé—*Aspidium viviparum* Féé
—*Polystichum heterolepis* Féé. Bud at tip
of leaf. Hooker & Baker (1868), Jenman
(1885), Maxon (1909).

XXIII. *Pteridium* Gleditsch

1. *P. aguilinum* (L.) Kuhn—*Pteris aguilina* L.
Buds on rhachis. Atkinson (1894), Clute
(1909), Engler & Prantl (1902), Goebel
(1887), Hofmeister (1857), Sachs (1882,
Schenk (1881).

XXIV. *Pteris* L.

1. *P. radicans* Christ. Buds on rhachis near apex,
and also at tips of pinnae. Kupper (1906).
2. *P. tripartita* Sw.—*Asplenium lineare* Pr. Buds
at tips of pinnae. Goebel (1928).

XXV. *Stenochlaena* J. Sm.

1. *S. sorbifolia* (L.) J. Sm.—*Acrostichum sorbi-*
folium L. Buds on rhachis in axils of pin-
nules. Kupper (1906).

XXVI. *Tectaria* Cav.

1. *T. cicutaria* (L.) Cop.—*Aspidium cicutarium*
(L.) Sw. Bulbils in axils of pinnae. Goebel
(1930).
2. *T. coriandrifolia*. Buds in axils of pinnae.
Eaton (1906).
3. *T. crenata* Cav.—*Aspidium platyphyllum* Pr.
Buds on rhachis usually near apex. Kupper
(1906).
4. *T. martinicensis* (Spr.) Cop.—*Aspidium fraxi-*
nifolium Schrad. Buds on rhachis in axils
of pinnules. Kupper (1906).
5. *T. plantaginea* (Jacq.) Maxon—*Aspidium*
plantagineum (Jacq.) Grieseb. Buds on
rhachis usually near apex. Kupper (1906).
6. *T. sparsiflora* (Hk.) Alston—*Phegopteris spar-*
siflora Sadebeck. Buds on lower surfaces of
leaves. Engler & Prantl (1902), Sadebeck
(1895).

XXVII. *Triphlebia* Bak.

1. *T. pinnata* (J. Sm.) Bak.—*Scolopendrium pinnatum* J. Sm. Bud at tip of elongated rhachis. Kupper (1906).

XXVIII. *Woodwardia* Sm.

1. *W. radicans* (L.) Sm.—*W. orientalis* Sw. Buds on rhachis at or near apex. Bower (1923), Druery (1896), Engler & Prantl (1902), Holm (1925), Hooker & Baker (1868), Sachs (1882), Schenk (1881).

Schizaeaceae

I. *Aneimia* Sw.

1. *A. radicans* Raddi—*A. caudata* Kaulf. Bud at tip of elongated rhachis. Hooker & Baker (1868), Kupper (1906).
2. *A. rotundifolia* Schrad. Bud at tip of elongated rhachis. Goebel (1902), (1905), (1930), Hooker & Baker (1868), Kupper (1906).
3. *A. Warmingii* Prantl. Bud at tip of elongated rhachis. Kupper (1906).

From Roots

Ophioglossaceae

I. *Botrychium* Sw.

1. *B. virginianum* (L.) Sw. Grevillius (1895).

II. *Ophioglossum* L. Campbell (1923), Goebel (1887), Holle (1875), Poirault (1893), Rabenhorst (1889), Sachs (1882), Stenzel (1858).

1. *O. Bergianum* Schlecht. Boodle (1899), Poirault (1892), Hooker & Baker (1868).
2. *O. capense..* Prantl (1884).
3. *O. coriaceum* A. Cunn. Prantl (1884).
4. *O. crotalophoroides* Walt.—*O. bulbosum* Michx. Hooker & Baker (1868).
5. *O. ellipticum* Hk. & Grev. Boodle (1899), Prantl (1884).
6. *O. Engelmanni* Prantl. (Plants in herbarium at University of Missouri).
7. *O. japonicum* Prantl. Prantl (1884).
8. *O. Luersseni* Prantl. Prantl (1884).

9. *O. lusitanicum* L. Hooker & Baker (1868), Prantl (1884).
10. *O. macrorrhizum* Kze. Poirault (1892).
11. *O. nudicaule* L. fil. Hooker & Baker (1868).
12. *O. pedunculosum* Desv. Goebel (1902), (1930), Hofmeister (1857), Holm (1925), Prantl (1884).
13. *O. pendulum* L. Boddle (1899).
14. *O. pusillum* Nutt. Eaton (1906).
15. *O. reticulatum* L. Prantl (1884).
16. *O. rubellum* Welw. Hooker & Baker (1868).
17. *O. vulgatum* L. Bower (1923), Engler & Prantl (1902), Goebel (1902), (1930), Hofmeister (1857), Holm (1925), Kerner & Oliver (1895), Petry (1914), Poirault (1891-92), Schenk (1881), West (1917), Wittrock (1883).

Polypodiaceae

I. *Adiantum* L.

1. *A. diaphanum* Bl. Birkenhead (1886).
2. *A. Moorei* Bak.—*A. amabile* Moore. Birkenhead (1886).

II. *Antrophyum* Klff.

1. *A. plantagineum* (Cav.) Klff. Goebel (1902), (1930).

III. *Asplenium* L.

1. *A. auritum*. Jenman (1885).
2. *A. cultrifolium*. Poirault (1893).
3. *A. fragrans* Sw.—*A. planicaule* Lowe. Birkenhead (1886).

IV. *Cheilanthes* Sw.

1. *C. Bergiana* Schlecht.—*Hypolepis Bergiana* (Schlecht) Hk. Birkenhead (1886).

V. *Diplazium* Sw.

1. *D. esculentum* (Retz.) Sw.—*Asplenium esculentum* Pr.—*Anisogonium esculentum*. Bower (1923), Goebel (1902), (1905), (1930), Holm (1925), Lachmann (1886), Poirault (1893), Rostowzew (1890).

VI. *Hecistopteris* J. Sm.

1. *H. pumila* (Spr.) J. Sm. Goebel (1930).

VII. *Platycerium* Desv.

1. *P. biforme*. Birkenhead (1886).
2. *P. bifurcatum* (Cav.) C. Chr.—*P. alcicorne* Desv.
Birkenhead (1886), Goebel (1905), Rostowzew
(1890), Watson (1886).
3. *P. grande* (A. Cunn.) J. Sm. Goebel (1930).
4. *P. Hillii* Moore. Goebel (1905), Rostowzew
(1890).
5. *P. stemaria** (Beauv.) Desv.—*P. alcicorne*. Birken-
head (1886), Goebel (1905), (1930), Rostowzew
(1890), Watson (1886).
6. *P. Wallichii* Hk. Poirault (1893).
7. *P. Willinkii* Moore. Birkenhead (1886), Goebel
(1930), Rostowzew (1890), Wittrock (1883).

By Apospory

Cyatheaceae

I. *Alsophila* R. Br.

1. *A. van Geertii* van Geert. Goebel (1907), (1908).
2. *A. tomentosa* (Bl.) Hk. Beyerle (1932).

II. *Dicksonia* L'Herit.

1. *D. Dayi*. Beyerle (1932).
2. *D. fibrosa* Col. Beyerle (1932).

Hymenophyllaceae

I. *Trichomanes* L.

1. *T. alatum* Sw. Atkinson (1894), Bower (1887).
2. *T. Kraussii* Hk. & Grev. Goebel (1905), Woronin
(1908).
3. *T. pyxidiferum* L. Bower (1887).
4. *T. trigonum* Desv.—*T. Kaulfussii* Hk. & Grev. Bower
(1894), Georgevitch (1910).

Osmundaceae

I. *Osmunda* L.

1. *O. regalia* L. Kohler (1919–20), Lawton (1932),
(1936), Manton (1932).

Parkeriaceae

I. *Ceratopteris* A. Brongn.

1. *C. thalictroides* (L.) Brongn. Beyerle (1932), Goebel
(1907), Kohler (1919–20).

* See *P. bifurcatum* (Cav.) C. Chr.

Polypodiaceae

- I. *Adiantum* L.
 - 1. *A. fulvum* Raoul. Beyerle (1932).
- II. *Anogramme* Link.
 - 1. *A. chrysophylla*. Beyerle (1932).
 - 2. *A. leptophylla* (L.) Link. Beyerle (1932).
- III. *Aspidium* Sw.
 - 1. *A. capense*. Beyerle (1932).
- IV. *Asplenium* L.
 - 1. *A. dimorphum* Kze. Goebel (1905).
 - 2. *A. nidus* L. Beyerle (1932).
 - 3. *A. platyneuron* (L.) Oakes. Lawton (1932).
 - 4. *A. serratum* L. Beyerle (1932).
- V. *Athyrium* Roth
 - 1. *A. Filix-femina* (L.) Roth—*Asplenium Filix-femina* Bernh. Atkinson (1894), Beyerle (1932), Druery & Bower (1884), Farmer & Digby (1907), Lawton (1932), Stansfield (1899).
- VI. *Blechnum* L.
 - 1. *B. capense* (L.) Schlecht.—*Lomaria capensis* Willd. Beyerle (1932).
- VII. *Cystopteris* Bernh.
 - 1. *C. fragilis* (L.) Bernh. Lawton (1932).
- VIII. *Davallia* Sm.
 - 1. *D. canariensis* (L.) Sm. Beyerle (1932).
- IX. *Diplazium* Sw.
 - 1. *D. melanocaulon* Brack. Beyerle (1932).
- X. *Drynaria* (Bory) J. Sm.
 - 1. *D. heraclea*. Beyerle (1932).
- XI. *Dryopteris* Adans.
 - 1. *D. marginalis* (L.) Gray—*Aspidium marginale* (L.) Sw. Lawton (1932).
 - 2. *D. pseudo-mas*—*Lastrea pseudo-mas*—*Nephrodium pseudo-mas*. Bower (1887), Digby (1905), Farmer & Digby (1907).
 - 3. *D. thelypteris* (L.) A. Gray. Beyerle (1932).
- XII. *Gymnogramma* Desv.
 - 1. *G. Hooker* J. Sm. Beyerle (1932).

XIII. *Hemionitis* L.

1. *H. palmata* L. Beyerle (1932).

XIV. *Nephrolepis* Schott

1. *N. biserrata* (Sw.) Schott. Beyerle (1932).

XV. *Notochlaena*—*Notholaena* R. Br.

1. *N. sinuata*. Kohler (1919–20).

XVI. *Phyllitis* Ludwig

1. *P. Scolopendrium* (L.) Newm.—*Scolopendrium vulgare* Sm. Farmer & Digby (1907), Kohler (1919–20).

XVII. *Pityrogramma*

1. *P. chrysophylla* (Sw.) Link—*Gymnogramma chrysophylla* Klff.—*G. Laucheana* Koch. Beyerle (1932), Goebel (1907).

XVIII. *Platycerium* Desv.

1. *P. bifurcatum* (Cav.) C. Chr.—*P. alcicorne* Desv. Kohler (1919–1920).
2. *P. Hillii* Moore. Kohler (1919–20).
3. *P. stemaria** (Beauv.) Desv.—*P. alcicorne* Desv. Kohler (1919–1920).

XIX. *Polypodium* L.

1. *P. aureum* L. Beyerle (1932), Goebel (1907), Kammer (1925–26).
2. *P. Fendleri* Eat. Beyerle (1932).
3. *P. loriceum* L. Beyerle (1932).
4. *P. musifolium* Bl. Beyerle (1932).
5. *P. punctatum* (L.) Sw.—*P. irioides* Poir. Steil (1921).
6. *P. vulgare* L. var. *elegantissimum*. Kohler (1919–20).

XX. *Polystichum* Roth.

1. *P. acrostichoides* (Michx.) Schott. Lawton (1932).
2. *P. aculeatum* (L.) Schott—*P. angulare* Pr. Atkinson (1894), Druery, Bower & Dyer (1884), Druery (1887).

XXI. *Pteridium* Gleditsch.

1. *P. aguilinum* (L.) Kuhn.—*Pteris aguilina* L. Atkinson (1894), Farlow (1888–89).

* See *P. bifurcatum* (Cav.) C. Chr.

XXII. *Pteris* L.

1. *P. cretica* L. Lawton (1932), Kammer (1925-1926).
2. *P. longifolia* L. Goebel (1907).
3. *P. sulcata* L. Steil (1919).
4. *P. tremula* R. Br. Beyerle (1932).

XXIII. *Woodwardia* Sm.

1. *W. virginica* (L.) Sm. Lawton (1932).

XXIV. *Tectaria* Cav.

1. *T. Maingayi* (Bak.) C. Chr.—*Campylogramme Trollii* Goebel. Beyerle (1932).

Schizaeaceae

I. *Aneimia* Sw.

1. *A. Dregeana* Kze. Goebel (1907).

By Induced Proliferation

Cyatheaceae

I. *Alsophila* R. Br.

1. *A. Van Geertii* van Geert. Bud from detached primary leaf. Goebel (1908).

Ophioglossaceae

I. *Ophioglossum* L.

1. *O. vulgatum* L. Buds from stem and root fragments. Poirault (1893).

Osmundaceae

I. *Osmunda* L.

1. *O. regalis* L. Bud from decapitated plant. Dopouloscheg-Uhlar (1911).

Polypodiaceae

I. *Adiantum* L.

1. *A. capillus-veneris* L. Buds from isolated primary leaves, from older leaves, from petioles and from rhizomes. Reinhold (1926).
2. *A. subcordatum* Sw.—*A. conicum* Vell. Buds from isolated primary leaves. Beyerle (1932).

II. *Aspidium* Sw.

1. *A. capense*. Buds from isolated primary leaves. Beyerle (1932).

III. *Asplenium* L.

1. *A. nidus* L. Bud from petiole of detached leaf. Beyerle (1932).

2. *A. platyneuron* (L.) Oakes. Buds from detached primary and secondary leaves and from detached roots. Lawton (1932).

IV. *Athyrium* Roth

1. *A. filix-femina* (L.) Roth. Buds from petioles. Also on young plants which have been decapitated. Beyerle (1932), Dopouloscheg-Uhlar (1911), Druery (1885), Hofmeister (1857).

V. *Cystopteris* Bernh.

1. *C. bulbifera* (L.) Bernh. Buds from fleshy leaves of bulbils. Heinricher (1896), (1899), (1900), Palisa (1900).
2. *C. fragilis* (L.) Bernh.—*C. alpina* Desv. Buds on decapitated plants, on isolated petioles and detached leaves. Dopouloscheg-Uhlar (1911), Heilbron (1910), Heinricher (1900).
3. *C. montana* (Lam.) Bernh. Buds from isolated petioles. Heinricher (1899), (1900), Palisa (1900).

VI. *Davallia* Sm.

1. *D. canariensis* (L.) Sm. Buds from isolated primary leaves. Beyerle (1932).

VII. *Diplazium* Sw.

1. *D. melanocaulon* Brack. Bud on isolated primary leaf. Beyerle (1932).

VIII. *Drynaria* (Bory) J. Sm.

1. *D. heraclea*. Buds from isolated primary leaves Beyerle (1932).

IX. *Dryopteris* Adans.

1. *D. filix-mas* (L.) Schott—*Lastrea filix-mas* Pr. Buds on base of fronds of decapitated plant. Druery (1896).
2. *D. Linnaeana* C. Chr.—*P. Dryopteris* Fée. New plants arise from internodes. Dopouloscheg-Uhlar (1911).
3. *D. marginalis* (L.) Gray—*Aspidium marginale* (L.) Sw. Buds from petiole of detached leaf. Lawton (1932).
4. *D. parasitica* (L.) O. Ktze.—*Nephrodium molle* R.

Br. Buds from callus of decapitated plant.
Doposcheg-Uhlar (1911).

5. *D. phegopteris* (L.) C. Chr.—*Phegopteris polypodioides* Fée. Buds from detached leaf of young plant. Brown (1918).
6. *D. thelypteris* (L.) A. Gray. Buds from isolated primary leaves. Beyerle (1932).

X. *Hemionitis* L.

1. *H. palmata* L. Buds from isolated primary leaves. Beyerle (1932).

XI. *Nephrolepis* Schott

1. *N. biserrata* (Sw.) Schott. Buds from isolated primary leaves. Beyerle (1932).

XII. *Platycerium*

1. *P. grande* (A. Cunn.) J. Sm. Buds from detached primary leaves. Kohler (1919–20).

XIII. *Polypodium* L.

1. *P. aureum* L. Buds from isolated primary leaves. Beyerle (1932), Goebel (1908).
2. *P. Fendleri* Eat. Buds from isolated primary leaves. Beyerle (1932).
3. *P. loricatum* L. Buds from isolated primary leaves. Beyerle (1932).
4. *P. musifolium* Bl. Buds from isolated primary leaves. Beyerle (1932).

XIV. *Pteris*

1. *P. serrulata*. Buds from decapitated plant. Doposcheg-Uhlar (1911).

XV. *Tectaria* Cav.

1. *T. Maingayi* (Bak.) C. Chr.—*Campylogramme Trollii* Goebel. Intermediate growths on isolated primary leaves from which buds arise. Beyerle (1932).

XVI. *Woodsia* R. Br.

1. *W. obtusa* (Spr.) Torrey. Buds from detached leaves and roots. Lawton (1932).

Schizaeaceae

I. *Aneimia* Sw.

1. *A. phyllitidis* (L.) Sw.—*A. densa* Link. Buds from isolated primary leaves. Beyerle (1932).

PROLIFERATION FROM LEAVES

References to vegetative reproduction from leaves of ferns are numerous. They often consist only of a brief notice that a fern reproduces in such a manner, no discussion of the origin or development of the new plant being given. Even when these are considered, the authors sometimes do not agree. In this review I have stated (or conjectured) the origin when possible.

Kunze, as early as 1849, stated that buds are often produced on fronds of ferns, especially on the rhachis near the base or the tip; also, that they are found on branch veins usually in the angles of pinnae or in the notches of the margins of fronds. He mentioned the bulbils formed on *Cystopteris bulbifera* (L.) Bernh. and *Asplenium bulbiferum* Forst.

Hofmeister (1857) was probably the first to describe the origin of buds on the fronds of ferns. In *Pteridium aquilinum* (L.) Kuhn (*Pteris aquilina* L.) buds arise on the dorsal portion of the petiole sometimes so close to the point of departure of the leaf from the stem that on superficial inspection they appear to originate from the stem. These buds arise from surface cells on the dorsal surfaces or margins of very young fronds long before the vascular bundles are differentiated from the remaining tissue. The division of the primordial cell proceeds in the same way as in the apical cell of the stem. When the development of the bud is slow, the marginal tissue closes over it almost completely. These buds may remain dormant for a long period. In *Dryopteris Filix-mas* (L.) Schott. (*Aspidium Filix-mas*) the buds are formed higher on the petiole. Roots are formed while the buds are still attached to the mother plant. Buds arise very near the base of the petiole in *Dryopteris spinulosa* (Müll.) O. Ktze. (*Aspidium spinulosum* Sw.) and *Matteuccia Struthiopteris* (L.) Todaro (*Struthiopteris germanica* Willd.). They originate very early in the ontogeny of the leaf, long before the differentiation of the lamina. In *Asplenium Belangeri* the buds are formed from superficial cells of the laminae. In all of the ferns studied by Hofmeister the buds originate from meristematic cells.

A detailed account of the origin of buds from the laminae of fronds was given by Heinricher (1878). He studied *Diplazium celtidifolium* Kze. (*Asplenium celtidifolium* Mett.), *Asplenium bulbiferum* Forst., *A. viviparum* (L. f.) Pr. and *A. Belangeri*; *A.*

bulbiferum was investigated more thoroughly than the others. The buds arise very early in the development of the leaf and not very far from the apex of the frond or of the pinnae. Each bud probably originates from a single superficial cell from which an apical cell is soon formed. In all of the ferns examined, the buds, after formation of the first frond, grow by means of a "three-sided" apical cell. The buds are united to the parent organ by the vascular tissue.

In many species, as in those just mentioned, proliferation is intimately connected with apical meristems. It is necessary, therefore, to summarize here the phenomena of apical growth as it occurs in ferns. Each meristem of the majority of ferns has its origin in a single cell called the apical cell or apical initial. Several types of apical cells have been distinguished by shape and the number of sides on which segments are formed. These are frequently referred to as "two-sided," "three-sided" and "four-sided," depending on the number of "cutting faces." Such terms are not entirely satisfactory. A "two-sided" apical cell may have a lens-shaped outer surface and a total of three surfaces, as in the leaves of *Camptosorus* (McVeigh 1936, Fig. 1), or may be cuneiform with five sides, as in *Riccardia* and some other liverworts. In the Marchantiales the apical cell is of the exact shape of the cuneiform "two-sided" cell but forms segments on four sides. Such cells are called "four-sided." A tetrahedral apical cell may have three "cutting faces," as in the stems of most ferns, or it may have four cutting faces, as in the roots of ferns. The former type is often referred to as "three-sided." The tetrahedral apical cell is sometimes called a triangular apical cell because each of its surfaces is triangular. The following terms designate both shape and method of segmentation and are self-explanatory: (1) biserrate lenticular, (2) biserrate cuneiform, (3) quadriseriate cuneiform, (4) triseriate tetrahedral, and (5) quadriseriate tetrahedral.

In 1881 Zimmerman reported a study of apical cells of adventitious buds of some ferns. He found a clearly recognizable apical cell in the buds of *Asplenium bulbiferum* Forst., *A. flabellatum* Kze., *A. Dregeanum* Kze. and in *Ceratopteris*. Neither he nor Heinricher (1878) succeeded in finding the earliest stages in the formation of buds and both were unable to determine with certainty whether the origin was in one or more epidermal cells.

Later, Heinricher (1881) succeeded in finding the very beginning of buds and in proving that they originate from single superficial cells which immediately form triseriate apical cells. The apical cell is usually the result of three divisions, but occasionally may result from two or four divisions.

Matouschek (1894) described the bulbils of *Cystopteris bulbifera* (L.) Bernh. but did not study their origin and development. Since they are not surrounded by broken and decaying tissue he concluded that they arise exogenously.

The origin, development and germination of the bulbils of *Cystopteris bulbifera* were investigated by Rostowzew (1894). These buds arise from single epidermal cells on the sides of the midrib where the lateral veins arise. A cell, considerably larger than those which surround it, occupies the middle of a small protuberance. Soon this cell divides in three directions and becomes tetrahedral. The whole primordium is a hemispherical protuberance in the center of which is the triseriate apical cell. The bulbil is attached to the parent leaf by a small stalk or base. While it is still very small, the first and second leaves are formed. They are not foliage leaves but scales which contain a large amount of food. The number of scales varies from two to seven. The fibrovascular strand of the bud is connected to the nearest bundle of the leaf. At the base of the bud near the attachment of the first scale the vascular bundle divides into two strands, one of which passes into the axis of the bud and the other into the scale. The buds appear as the leaves develop, and, since the leaves continue to grow throughout a long period of time, buds of various ages are found on a single leaf. The epidermal cells concerned in the origin of the new plant are probably not highly specialized. Rostowzew stated that the mother cells of stomata (guard cells) may be mistaken for bud primordia since both arise from single epidermal cells. This would indicate that the cells of the leaf are still meristematic. Sadebeck's account in Engler and Prantl (1902) agrees with that of Rostowzew.

Atkinson (1894) described and pictured some early stages in the formation of adventitious buds of *Asplenium bulbiferum* Forst. Each bud arises near a vein. Sections of pinnules at the point of origin of buds show a tissue on one side of the vein composed of mesophyll and epidermal cells which contain abundant protoplasm. The mesophyll cells in this region are smaller than in other parts of

the leaf. Size and contents of these cells indicate that they are meristematic. Sections of very young buds show what appears to be an apical cell, perhaps that of the stem. Atkinson considered it incorrect to say that the bud originates from a single epidermal cell or even from the epidermis, since the mesophyll cells between the epidermis and the vascular bundle also undergo changes. They elongate, lose their abundant protoplasmic content and become scalariform tracheids in connection with those of the bundles of the pinnae. However, Atkinson considered it possible that the bud is derived from a superficial cell of the young leaf before the differentiation of the epidermis.

In *Angiopteris evecta* (Forst.) Hoffm. a somewhat different means of propagation was described by Raciborski (1902). Buds are formed where the margins of the stipules pass into the leaf base. They remain dormant until the leaf bases become separated from the main axis and encounter an environment suitable for growth. Raciborski did not discuss the origin of these buds; they probably originate early in the ontogeny of the frond while the tissue is still meristematic.

In other ferns, such as *Woodwardia radicans* (L.) Sm. and *Leptochilus heteroclitus* (Pr.) C. Chr. (*Chrysodium flagelliferum* Mett.), buds are produced at the tips of elongated leaves. These buds come in contact with the soil and there root and form new plants. Sadebeck in Engler and Prantl (1902) did not discuss the origin of such buds, but since there is a meristematic region at the tip of a fern leaf, they presumably arise from unspecialized cells. As before mentioned, *Pteridium aquilinum* (L.) Kuhn, *Dryopteris Filix-mas* (*Aspidium Filix-mas*) and *Dryopteris spinulosa* (Müll.) Kze. produce adventitious buds on the petioles. According to Sadebeck, these buds arise very early before development of the lamina and before differentiation of the tissues has begun. He reported other ferns which reproduce vegetatively but did not discuss the origin of the new plants.

Goebel (1905) was the first to describe the development of buds which arise at the tips of elongated leaves. He investigated the formation of buds in *Adiantum Edgeworthii* Hk. to determine whether the new plant really grows out of the tip of the leaf or is laid down near the biserrate lenticular (two-sided) apical cell of the parent leaf. In leaves which reproduce vegetatively, the apical

cell becomes divided by an anticlinal wall into two similar triseriate tetrahedral cells. One of these becomes the apical cell of the bud formed at the tip of the leaf while the other becomes divided in an irregular manner. The first leaf originates on the convex side of the parent leaf from a position near the new stem apex; roots are formed endogenously. According to Goebel, elongation of the parent leaf into a flagellum occurs only after formation of the bud. If this is true the increase in length must result from intercalary growth, since the apical meristem has already become organized into a bud; in this respect the leaves differ from those of most ferns in which the increase in length is due to apical growth.

Kupper (1906), a student of Goebel, also investigated the origin of the adventitious buds of *Adiantum Edgeworthii*. His results agree with those of Goebel but are somewhat more complete. The second and third leaves as well as the first do not originate from segments of the apical cell of the stem but from meristematic cells at some distance from the apex of the shoot. The first roots arise endogenously from the convex side of the tip of the parent leaf; the later ones from the stem of the bud. In leaves which do not reproduce vegetatively the apical cell after formation of the last lateral pinnule changes to marginal growth and forms a terminal pinnule. *Adiantum caudatum* L., *A. lunulatum* Burm. and *A. capillus-junonis* Rupr. form buds in a similar manner.

In most respects the formation of buds at the tips of leaves of *Asplenium achilleifolium* (Lam.) C. Chr. (*A. prolongatum* Hk.) is similar to that in species of *Adiantum*. The apical cell of the leaf is divided by an anticlinal cross wall which passes obliquely from side wall to side wall. The portion of the apical cell toward the ventral side of the frond becomes the apical cell of the shoot and forms segments on three faces; the other portion becomes divided into smaller cells. The convex or dorsal portion of the tip of the leaf grows much larger than the concave or ventral side and causes the whole apical cell group to be moved to the ventral side of the leaf. This may occur before the division of the original apical cell of the parent leaf. The first roots appear on the front and sides of the swollen tip. They often originate in the first layer under the epidermis. The first leaf is distal to the shoot apex; it originates from the thickened portion of the mother leaf and not from a segment of the apical cell of the stem. The origin of the

second and third leaves was not determined. The production of buds is accompanied in some forms of this fern by a reduction in the number of pinnules and sometimes even the complete suppression of pinnules.

According to Kupper, the buds formed at the tips of the elongated fronds of *Aneimia rotundifolia* Schrad., *Camptosorus rhizophyllus* (L.) Link (*Scolopendrium rhizophyllum* Endl.), *Fadyenia Fadyenii* (Mett.) C. Chr. (*Fadyenia prolifera* Hk.) originate in much the same manner. The apical cell of the leaf very early becomes divided and there results a continuous row of marginal cells; these then become divided into more or less isodiametric cells and at the same time the tip of the leaf becomes considerably thickened. The apical cell of the stem is formed on the highest part of the thickened tip. The first leaves originate from the meristematic tissue at the tip of the parent leaf and usually appear before the apical cell of the new stem is formed. The number of leaves which arise independently of the stem apex varies in different ferns. The first roots arise endogenously around the sides and ends of the enlarged tip. Kupper did not investigate the origin of the leaves and roots of *Fadyenia Fadyenii* but expressed the opinion that it is similar to that in the other species described. The first several leaves of these ferns do not reproduce vegetatively.

In *Trichomanes pinnatum* Hedw. Kupper found that the buds arise on both sides of the elongated rhachis in place of pinnules. The buds, like the pinnules, originate from cells of the marginal row.

Asplenium oceanicum C. Chr. (*A. obtusilobum* Hk.) and *A. Mannii* Hk. produce two types of leaves; foliage leaves which do not form buds, and stolons which form buds. According to Kupper, the apical cell of the stolon leaf is not used up in the formation of buds but they are formed only in its vicinity. In *A. Mannii* and *Trichomanes pinnatum* Hedw. the buds originate from marginal cells, while in *A. oceanicum* the buds are formed on the upper side of the stolon. In these ferns the first leaf arises independently of the stem apex.

Bally (1909) found that the buds on the leaves of *Ceratopteris thalictroides* (L.) Brongn. arise exogenously and he thought that each probably originates from a single epidermal cell. The youngest stage he found consisted of two cells. By the later division of

one of these cells a triseriate-tetrahedral apical cell is formed. The other cell becomes the apical cell of the first leaf. Later leaves arise from segments of the apical cell of the stem.

According to Haberlandt (1914), the buds formed on the fronds of *Asplenium bulbiferum* Forst., *A. viviparum* (L.f.) Pr. and *Ceratopteris thalictroides* (L.) Brongn. have their origin in the protodermal layer while the leaf is still quite young. A single element of the protoderm gives rise by appropriate divisions to a triseriate cell which is the apical cell of the stem of the new bud. Since the protoderm is one of the primary meristems, such buds evidently arise from unspecialized cells.

Bower (1923) reported the production of adventitious buds on the leaves of several ferns. Sometimes the buds are formed on the upper surface of the lamina (*Asplenium bulbiferum* Forst.) and sometimes on the lower surface (*Asplenium viviparum* (L.f.) Pr., *Diplazium celtidifolium* Kze. and *Dennstaedtia rubiginosa* (Klf.) Moore). The buds often arise near the bases of the pinnae, as in *Cystopteris bulbifera* (L.) Bernh. and *Woodwardia radicans* (L.) Sm. Bower stated that in some ferns the buds have been traced to single superficial cells, which renew activity; he did not discuss this further. He said that in *C. bulbifera* and *W. radicans* the production of sporophytic buds is related to the arrest of sori.

McVeigh (1934, 1936) and Yarbrough (1936) described the origin of buds in *Camptosorus rhizophyllus* (L.) Link. Both agree that the young plant originates from meristematic cells. According to Yarbrough, the apical cell becomes divided very early so that the marginal row of cells is continuous around the tip. Later as the tip thickens the marginal cells are moved higher and extend over the tops of the swellings; in the meantime they have become divided, although their outlines can be detected for some time. A cell in the highest part of the swelling enlarges and undergoes two divisions by which the stem apical cell is formed. On the contrary, McVeigh found that in those leaves which reproduce vegetatively the apical cell persists and takes part in the formation of the new plant; the first leaf is formed by the continued growth of the apical cell of the parent leaf; the stem arises in the meristematic part of the leaf tip, probably from one of the segments of the apical cell of the parent leaf; the roots arise endogenously from the meristematic cells of the leaf tip. Those leaves in which the apical cell loses

its identity, by becoming divided so that the marginal row of cells is continuous over the apex, do not proliferate.

PROLIFERATION FROM ROOTS

Proliferation from roots was mentioned as early as 1857 by Hofmeister who reported its occurrence in *Ophioglossum vulgatum* L. and *O. pedunculosum* Desv. Among the early workers who considered reproduction by roots but did not discuss the origin of the buds are Stenzel (1858), Van-Tieghem (1870-71), Prantl (1884), Jenman (1885) and Birkenhead (1886). This phenomenon is not nearly so common as proliferation from leaves. Most of the ferns which develop buds on the roots are included in two genera, *Platycerium* and *Ophioglossum*. In some species it is a very effective method of reproduction. Poirault (1891) thought that *O. vulgatum* was always propagated by such buds because he had never observed a prothallus, and Rostowzew (1890) noted that *Diplazium esculentum* (Retz.) Sw. (*Asplenium esculentum* Pr.) seldom formed fertile fronds. I have observed as many as eight plants of *O. Engelmanni* Pr. (growing wild near Columbia, Missouri) all connected by the root system.

Rostowzew (1890) made a detailed study of the origin of buds formed on roots. He investigated the transformation of tips of roots into sprouts in *Diplazium esculentum* and *Platycerium alcicorne* Desv. [*P. bifurcatum* (Cav.) C. Chr. and *P. stemaria* (Beauv.) Desv.] and observed such transformations in other species of *Platycerium*. The roots in the two species investigated have the usual quadriseriate tetrahedral apical cell covered by a well developed root cap. The apical cell itself becomes the apical cell of the new shoot; it ceases to cut off segments on the side of the root cap and as a result of this becomes triseriate like the apical cell of the stem. This cell and its segments continue to divide and soon break through the root cap. Very early leaves and roots are differentiated, often even before the bud breaks through the root cap. The vascular tissue of the root passes directly into the shoot and gradually develops the characteristics of the vascular anatomy of the stem. Old roots as well as young roots and lateral roots as well as tap roots may be changed into shoots. In *Diplazium*, usually the tips of the main roots are transformed but sometimes also those of the lateral roots. According to Rostowzew a lateral root primor-

dium may become transformed into a bud before it breaks through the cortex; such buds appear to arise laterally on the root instead of from the tip.

Probably the formation of buds from roots is more generally known in *Ophioglossum vulgatum* L. than in any other fern. Van Tieghem (1870-71) reported that buds may develop from the root tip as in the species just described or from any point on the root. According to Beyerinck* (1886) the apex of the root is transformed into the apex of a leafy stem. The investigations of Rostowzew (1891, 1892) and of Poirault (1891, 1892, 1893) do not confirm the opinions of these earlier students. Both Rostowzew and Poirault describe the formation of the bud close to the extremity of the root but consider it a lateral production. The root never loses its cap but continues its course as before. Rostowzew (1891) and Poirault (1893) describe in detail the formation of buds from the roots of this fern. The quadriseriate tetrahedral apical cell of the root is usually easy to recognize but at the time of bud formation this becomes more difficult because segmentation occurs more slowly on one side than on the others. Each segment is divided first by a curved partition which is not visible in a longitudinal section; then by a wall parallel to the surface of the root, the segment is divided into an inner and an outer cell. The outer cell, which gives rise to the young plant, becomes divided into two superposed layers. That nearest the terminal cell of the root is transformed by three successive inclined walls into the apical cell of the stem, while the other layer gives rise to the first leaf. This primordium enlarges and finally breaks through the cortex. Other leaves are formed from the stem apex. At the same time, the apical cell of the parent resumes segmentation, which results in the further separation of the root tip from the bud. Buds which originate as just described are produced only when the root has attained a certain length.

Buds may be produced on fragments of stems and roots kept in a moist environment. According to Poirault (1892) these buds originate endogenously from the cortex; he did not give a detailed account of their origin. Rostowzew had earlier reported the formation of such adventitious buds. Goebel (1902, 1930) also discussed the formation of buds on the roots of this species and of *O.*

* See Poirault (1890)—Ann. Sci. Nat. Bot. VII, 18: 113-256.

pedunculosum Desv. He considered it possible that primordia of branch roots are formed first in the fragments; these root primordia are transformed into buds before breaking through the cortex and thus appear as lateral buds which have been formed directly from the cortex. This seems improbable, for these plants do not normally form lateral roots. Goebel thought the place of origin of the lateral buds was not definitely fixed. This should be investigated.

Petry (1914) stated that *Ophioglossum pendulum* L. reproduces vegetatively by buds from roots just as does *O. vulgatum*, and that the development of the buds agrees in all important respects with that in *O. vulgatum* described by Rostowzew.

INDUCED PROLIFERATION AND APOSPORY

Apospory occurs in some species naturally, in others also after artificial stimulation, the results being similar. Because apospory and the artificial production of sporophytic buds so often result from the same stimulus, they are here discussed together. Very few investigators have determined the exact origin of the proliferated structures.

Hofmeister (1857) reported the production of adventitious buds on the bases of detached petioles of *Athyrium Filix-femina* (L.) Roth (*Asplenium Filix-femina*) which had been kept in a moist atmosphere for a long time. This fern exhibits an unusual ability to propagate itself. Apospory was first reported by Druery and Bower (1884) in this species and in *Polystichum angulare* var. *pulcherrimum* Padley. Bower found that in *A. Filix-femina* var. *clarissima* no mature sporangia or spores are produced; the thalli are developed either from the cells of the wall of the sporangium or from its stalk. In the *Polystichum* mentioned the prothalli may be simple vegetative outgrowths from the apices of pinnules, from arrested sporangia, or from the base of a sorus.

In 1899 Stansfield investigated the production of adventitious growths on isolated immature fronds of *Athyrium Filix-femina* var. *uncogglomerata* which were kept for some time in a humid atmosphere. The result was the development of a meristematic tissue which gave rise to gemmae or bulbils and to prothalli. The prothalli produced plants both by apogamy and amphimixis.

Apospory was reported in *Trichomanes alatum* Sw. and *T.*

pyxidiferum L. by Bower (1887). In these ferns apospory is accompanied by partial arrest of spore production. In *T. alatum* outgrowths may also occur from the tips of pinnae. Bower (1894) described briefly the production of filamentous outgrowths and gemmae from single marginal or superficial cells of *T. trigonum* Desv. (*T. Kaulfussii* Hk. & Grev.) grown under moist shady conditions.

Heinricher (1899) discussed the ability of the bulbils of *Cystopteris bulbifera* (L.) Bernh. to form "Regenerationsknospen." Earlier (1896) he had observed that old, partly decayed bulbils often gave rise to plants. This led to experiments with portions of bulbils. The buds are produced on the ventral portions of the fleshy leaves of the bulbils near the point of their insertion. They immediately develop foliage leaves instead of fleshy storage leaves like those which compose the bulbils. When the storage leaves are cut longitudinally into halves a single bud is formed on the basal portion of each. If they are cut crosswise two buds may arise on the basal half, one on each flank, while the terminal half forms none. Such buds have not been found on bulbils of which the vegetative point is intact. Heinricher did not discuss the origin or development of the "Regenerationsknospen."

According to Heinricher (1899, 1900) sporophytic buds are produced on the isolated basal portions of fronds of *Cystopteris montana* (Lam.) Bernh., *C. fragilis* (L.) Bernh. (*C. alpina* Desv.). Buds are produced on old as well as young leaves. Heinricher did no histological work to determine their origin.

Palisa (1900), a student of Heinricher, investigated the origin and development of the "Regenerationsknospen" of species of *Cystopteris*. In *C. bulbifera* and *C. montana* the buds arise from epidermal cells. Any epidermal cell on the ventral side in the vicinity of the leaf base possesses the ability to proliferate. The buds arise not from a single epidermal cell but from several which together divide and form a bud. An apical cell is formed in the new outgrowth, sometimes directly by the first division of an epidermal cell but more often after other divisions have taken place. From a single proliferation several buds may arise.

Doposcheg-Uhlar (1911) investigated the production of proliferations by embryos from which the stem tip had been removed. Plants with from two to five leaves were used. In *Athyrium Filix-*

femina (L.) Roth and *Dryopteris parasitica* (L.) Kze. (*Nephrodium Molle* R. Br.) buds originate exogenously from the callus formed on the cut surface. The apical cell of the new leaf is soon established. In *Osmunda regalis* L. and *Pteris serrulata* in addition to the buds formed exogenously from callus tissue others may arise endogenously. In *O. regalis*, Doposcheg-Uhlar described the production of a bud at the end of a vascular strand 8–10 layers of cells under the callus. In *P. serrulata* the buds arise exogenously but may also arise from the parenchyma of the cortex. Doposcheg-Uhlar experimented on internodes of older plants and obtained results similar to those with decapitated embryos. In *Dryopteris Linnaeana* C. Chr. (*Phegopteris Dryopteris* Féé) the parenchyma cells of the cortex start dividing, break through the epidermis and differentiate into a new plant. Internodes of *Cystopteris fragilis* (L.) Bernh. may form new plants from the callus which give rise to the first leaf and stem. Buds may arise also from the cortex as in *Dryopteris*.

According to Reinhold (1926) *Adiantum capillus-veneris* L. surpasses all other fern species in ability to produce regenerative outgrowths. These are formed from isolated primary leaves, from both fertile and sterile leaves of older plants and even from petioles and segments of the rhizome. Outgrowths sometimes arise on the surface of leaves of young plants. Near these arise structures which sooner or later give rise to vegetative points or shoots from which new plants develop. Ordinary epidermal cells are capable of division and give rise to the new growths. Those on the under side and on the edge of the leaf possess this ability to the greatest extent. Sometimes attached leaves put forth buds. No prothalli were observed on the isolated plant parts investigated. The other species of *Adiantum* investigated by Reinhold formed no adventive growths.

Beyerle (1932) investigated regeneration from isolated primary leaves of 57 species of 36 genera under different conditions of temperature, light and moisture. Of these, 34 species produced adventive structures. They were of various kinds, as follows: (1) sporophytic buds; (2) undifferentiated growths; (3) prothalli; (4) structures intermediate between gametophyte and sporophyte. In some species the structures arose irregularly distributed on the leaf; in others they were usually found on the bases or lateral margins of

the leaves. On those which generally gave rise to prothalli the new structures were most often found on the apical margins of the leaves near the ends of veins. According to Beyerle, the new structures formed by isolated primary leaves have their origin in epidermal cells.

DISCUSSION AND SUMMARY

Proliferation occurs in many species of ferns: (1) normal vegetative reproduction from leaves has been reported in 8 families, 35 genera and 197 species; (2) vegetative reproduction from roots in 2 families, 9 genera and 34 species; (3) production of sporophytes as the result of artificial stimuli in 5 families, 20 genera and 32 species; (4) apospory in 6 families, 30 genera and 51 species. Histological studies have been made of the origins of the proliferations in some species. These studies indicate that the plants which are produced normally from the leaves have their origin in unspecialized cells at the tip or from epidermal cells. Whether much differentiation has taken place in the epidermal cells concerned or whether the plants originate from superficial cells of very young leaves is not known. It may be that the new plants are initiated before the superficial cells have become much differentiated, *i.e.*, from the protoderm. The plants produced from roots arise from cells of the apical meristem and possibly from cells of the cortex. (Although it has been reported that buds arise from the cortex of fragments of roots of some species no detailed account of their origin has been given. A reinvestigation of this work is desirable.) Plants which are produced as a result of artificial stimuli have been found to originate from epidermal cells, parenchyma cells, callus cells and meristematic cells. In general, the cells concerned in proliferations of ferns seem to be unspecialized or not highly specialized; at least no great dedifferentiation would seem to be involved.

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THE BOTANICAL REVIEW

VOL. III

OCTOBER, 1937

No. 10

BACTERIOPHAGE IN RELATION TO PLANT DISEASES*

H. KATZNELSON

State College of Washington

I

The name bacteriophage was applied by d'Herelle (28) to an agent which causes transmissible lysis or dissolution of bacteria. The suffix "phage" means to eat but in this case it means to live or to develop at the expense of; hence, bacteriophage is an entity capable of developing at the expense of bacteria.

During the past twenty years few discoveries in bacteriology have aroused such interest and initiated such prolific experimentation as has this one of a substance which, with a suggestion of the magic and the supernatural of by-gone days, causes living things to disappear in a manner which is to this day a mystery. Nor is it difficult to understand why this discovery stimulated such interest and speculative enthusiasm. What could be more effective, for example, than the injection of this lytic substance into a patient suffering from typhoid? The principle would be disseminated through the body, the typhoid organisms destroyed, and the patient cured. Analogous to such a case might also be the treatment of plants suffering from a bacterial disease. Further work with "phage" has resulted, however, in the realization that such possibilities and activities are not quite so simple and straightforward as originally considered; that many factors militate against effective therapeutic and prophylactic use of the lytic agent. Some of these factors will be elucidated subsequently.

The lytic principle was discovered by Twort (72) in his search for vaccine virus and by d'Herelle (28) in the fecal discharges of dysentery convalescents. Some writers refer to bacteriophagy as the "Twort-d'Herelle phenomenon," although d'Herelle's extensive

* Published as Scientific Paper No. 360, College of Agriculture and Experiment Station, State College of Washington.

and stimulating work has resulted, generally speaking, in the identification of the phenomenon with his name. Since 1917 the study of the bacteriophage and its behavior has progressed in leaps and bounds with the result that today the literature on the subject is truly voluminous.

The bacteriophage is as widely distributed in nature as are bacteria and in fact considerable difficulty is often experienced in isolating bacteria entirely free from some associated bacteriophage. Rivers, sea-water, wells, reservoirs, sewage, soil, plant material, milk products, animal organs, and even bacterial cultures have all yielded lytic principles (43).

In fluid media it manifests its activity by clearing a turbid culture, on solid media clear areas (plaques) on confluent bacterial growth are produced; yet the mechanism of such clearing is as yet not completely understood. Increase in amount of active or "virulent" bacteriophage occurs exclusively in the presence of living, actively multiplying bacteria; hence, in a medium and at a temperature which are optimum for the development of the homologous organisms (71). It has been demonstrated by numerous workers that bacteriophage is active even at a dilution of 10^{-11} , although such activity depends on the age of the agent, age of the bacterial cultures used, and on various other factors.

Bacteriophage is filtrable through candles of porcelain and diatomaceous earth because of its minute size (1.2-75 $\mu\mu$) (66). Passage through semipermeable membranes depends on the charge of both phage particles and the membrane, surface tension, adsorption, and related phenomena. For example, the basic filters of Kramer (52) held back neutral solutions of phage, whereas acidified phage was retained even by ordinary Berkfeld filters (14). The principle is readily absorbed by both living and dead susceptible bacteria and by various organic and inorganic colloids, as well as by positively and negatively charged substances. The particles of the lytic principle are charged but the nature of this charge is still debatable because of the contradictory results obtained to date. It has been concluded by many that the lytic substance is in general less resistant to chemical and physical agents than bacterial spores, but more resistant than the vegetative forms of bacteria. It has antigenic properties since it is capable of producing specific antibodies on injection into experimental animals. The sera obtained

by this means are capable of neutralizing its activity (43). Opinion is now against the unicity of bacteriophage as claimed by d'Herelle (29). There evidently are distinct differences in phages as borne out in part at least by their antigenic specificity. In many cases a bacteriophage is specific to only one strain or species of bacteria, although in other cases, as with polyvalent phages, specificity is not so distinct (14).

A very interesting and important phenomenon connected with bacteriophagy is the development of resistant or non-susceptible cultures, which may take place *in vivo* and *in vitro*. After bacteriophagy is complete, certain of the bacteria in the culture have not been destroyed and are evidently resistant to the attack of this agent. As a result they begin to multiply in the apparently cleared fluid culture and produce turbidity once more. D'Herelle (29) states that certain bacteria, as typhoid and dysentery organisms, produce susceptible strains only, others, as coli and root nodule organisms (*Rhizobia*), produce both susceptible and non-susceptible strains. He calls the former homogeneous and the latter heterogeneous. The resistant cultures are markedly different in morphology, colony formation, fermentative powers, antigenic structure, and "virulence" from the original forms. From the point of view of virulence or ability to cause infection such resistant forms are very important. In many cases (14) the resistant forms are more "virulent" than the original organisms, although in some cases virulence is diminished or completely lost. Obviously, if an increase in virulence results after bacteriophagy, then the therapeutic or prophylactic value of the bacteriophage is open to serious criticism. Where homogeneous species are considered, however, there is a possibility that the bacteriophage may be truly efficacious in combating disease.

One of the most controversial points in the study of bacteriophagy is the nature of the lytic agent. D'Herelle (29) claims that it is an autonomous living agent, an ultramicroscopic infectious disease of bacteria. Kabeshima (48) first suggested that the bacteriophage was not animate but enzymatic in nature and derived from leucocytes. Bordet and Ciua (11, 12) also believe it is inanimate but produced by the bacterial cell, whereas Hadley (42, 43) suggests that it may be a filtrable stage in the life cycle of bacteria. Generally, it seems that the inanimate nature of the bacteriophage is favored (79). The recent work of Stanley (68) and others in

establishing the protein nature of tobacco mosaic virus may soon lead to analogous results with bacteriophage; hence, in a definite step forward in the solution of this problem.

The foregoing rapid survey of the bacteriophage and its behavior may have raised a question regarding the possible relationship between bacteriophage and filtrable viruses. According to d'Herelle's (29) views, the lytic principle must be considered a virus disease of bacteria. The analogies between the two agents are numerous (79). "Both appear to be minute particulate bodies invisible under the microscope, filtrable, uncultivable upon lifeless media, propagated only in the presence of living cells, relatively specifically adapted to certain cells, resistant and sensitive to the same physical and chemical agents, and affecting cells by stimulating or destroying them or both. In fact, almost every general characteristic of the viruses of man and animal is exhibited by bacteriophage." It is interesting to note that Bewley (8) considers bacteriophage and mosaic virus of tomato as identical. As far as the nature of these two agents is concerned, however, particularly with respect to their inanimate or animate nature, controversy is still great, although it seems that evidence is generally increasing that they are inanimate entities (79).

II

The earliest investigators in the field of bacteriophage, foremost of whom was d'Herelle, emphasized the medical aspects of the problem. They considered bacteria, such as typhoid, dysentery, and cholera organisms, and studied under the driving stimulus of d'Herelle's investigations and theories the possible therapeutic and prophylactic propensities of this lytic principle. When other workers began to isolate phages from water, sewage, soil, milk products, plants, and so on, the question arose as to the possibility that the lytic agent was active because of its evident ubiquity against organisms other than those associated with animals. The question was quite conclusively answered by later research work and will be dealt with subsequently, particularly in relation to bacterial plant diseases.

METHODS OF STUDY

Chester (21) discusses very thoroughly the problems involved in the isolation of bacteriophage from plants. Such isolation may

be effected in a number of ways. Sap may be expressed from the tissues, as roots, stems, leaves and galls, filtered through sterile candles and tested against the particular bacterial pathogen under consideration. Since this pathogen may be homogeneous or heterogeneous, it is necessary to test the filtrate against a number of strains of the pathogen studied. These strains may be obtained by plating out stock cultures of the organism and picking colonies or by plating out strains isolated from the plant lesions, although there is some danger in the latter method of obtaining at the same time an associated bacteriophage. If lysis does not occur after about five to ten serial passages, one may conclude that the agent probably is absent.

Again, the tissues under consideration may be crushed in a mortar, using sterile water or bouillon. The resulting mixture is filtered and tested as above. Another method is to wash the tissue free of foreign matter, sterilize the surface by a flame or cut with a sterile knife and remove the central portion. This is placed in a sterile mortar, 10 c. cm. of a fluid medium containing the homologous bacteria are added, the mixture is macerated, transferred to a sterile test tube and incubated for several days. The phage, if present, develops at the expense of some of the bacteria and becomes more active, and on filtration and subsequent serial passages manifests itself more rapidly and more powerfully than in the unincubated filtrates. Flaming tissue surfaces prevents contamination and is superior to chemical disinfectants which may enter the tissue and inhibit the development of both the phage and the bacteria.

Another method often resorted to and used to some extent by Gerretsen *et al.* (40), Demolon and Dunez (26) and Vandecaveye and Katzenelson (75) is to place the tissue (aseptically) on a plate streaked with the organism under investigation. The disappearance of growth in the vicinity of the tissue indicates the presence of a lytic agent, although it may not be a true bacteriophage. To be considered a true phage, a lytic agent must cause lysis in fluid cultures, produce plaques on agar plates, and be transmissible from agar and from fluid cultures (53). Such premises are necessary since Laird (53), Demolon and Dunez (26) and the author have noted the presence of a lytic principle in nodules of legume plants, which decreased in activity with each passage until it no longer

caused dissolution; hence, it was not transmissible and, therefore, not a true bacteriophage. A review of the literature on the bacteriophage in relation to phytopathogenic bacteria has brought out the fact that a number of investigators, especially the earlier ones, have used inhibition of bacterial activity as a manifestation of bacteriophage action. Now, whereas it may be entirely possible that they were dealing with a true phage, the criticism that the agent may not have been a bacteriophage is still valid and justifiable. Hence, inhibition alone should not be used as a criterion of bacteriophagy.

It has been pointed out previously that in general the most favorable medium, temperature and reaction for bacteriophagy are those which are optimum for the particular organism being studied. For more detailed information on methods, the reader is referred to the work of Laird (53), Chester (21) and Dufrenoy (34).

BACTERIOPHAGY AND BACTERIAL DISEASES OF PLANTS

Gerretsen *et al.* (40) were the first investigators to isolate a lytic principle from plants. They obtained a lytic agent active against the root nodule organisms from roots, stems and nodules of clover, lupine and serradella and from garden and field soil but not from forest soil. Their lytic agent resisted desiccation (two months) and a temperature of 60–65° C. for 15 minutes. It passed through collodion membranes and was eight times more resistant to ultraviolet light than the legume bacteria. Since the work of these investigators, numerous isolations of bacteriophages for the Rhizobia have been made (44, 27, 53, 26, 75). In 1924 Mallman and Hemstreet (55) obtained an inhibitory substance from cabbage rotted by a fluorescent organism. They did not demonstrate actual lysis but did obtain marked inhibition of growth at very high dilutions and were able to increase the potency of their principle by serial passages to 10^{-11} . Coons and Kotilia (23) prepared sterile filtrates from decaying carrots (soft rot) and on testing these against *Erwinia carotovora*¹ they obtained inhibition of growth after three days at 25° C. Similar results were obtained against *Erwinia atroseptica* with filtrates from soil drenched with heavy suspensions of pure cultures of this organism and with filtrates of river water against *Erwinia atroseptica*, *Phytomonas tumefaciens* and *Erwinia*

¹ Bergey's system of nomenclature is used throughout this paper.

carotovora. In addition, they isolated an inhibiting principle from cabbage, active against *Phytomonas tumefaciens*, although Israilsky (45, 46) made perhaps the most intensive study of the phage in relation to the crown-gall organism. Again, the potency of their filtrates could be increased by serial transfers to cause inhibition at a dilution of 10^{-8} . Temperature studies over a range of 7.8 to 36.1°C . showed that the optimum temperature range for lysis was 16.8 to 23.4°C ., which corresponds fairly closely to the optimum temperature range for the organism concerned. Moore (6) obtained a lytic principle from the juice of diseased tobacco leaves, active against *Phytomonas tabaca* (causal agent of wildfire disease of tobacco). He demonstrated lysis but could never obtain complete dissolution. The agent was definitely specific for living wildfire bacteria and was resistant to considerable heating and long storage.

Phytomonas tumefaciens, the crown-gall organism, has been the subject of numerous studies in relation to bacteriophage. Israilsky (45) demonstrated a phage in plant tumors experimentally produced in *Beta vulgaris*. From nine different isolations he obtained two strains of the bacterium susceptible to phage. After 12 serial passages the lytic agent was active at a dilution of 10^{-10} and was thermostable from $55\text{--}70^{\circ}\text{C}$. Later (46), however, he found bacteriophage in stock cultures of this bacterium; therefore, his previous results are open to the criticism that in producing the galls he injected along with the organism an associated bacteriophage. Healthy beets yielded no lytic agent. D'Herelle and Peyre (30) also pointed out that stock cultures of *Phytomonas tumefaciens* may or may not contain an associated phage. They claimed, too, that it is only such a bacteriophage-bacterium association which can cause crown-gall but they did not prove their theory conclusively. Later (18, 49, 50), Brown and Quirk, and Kauffmann came to an opposite conclusion from their experimental work. Brown and Quirk (18) also isolated bacteriophage from the juices of galled plants (castor-bean) but found that at high dilution bacteriophage stimulated bacterial growth and tumor formation, though at still higher dilution it retarded bacterial development *in vitro* and *in vivo*. D'Herelle (29) has found, too, that high dilutions of bacteriophage may stimulate bacterial growth. In fact, it has been quite generally noted that prior to bacteriophagy there is a marked acceleration of multiplication of bacteria (14). Muncie and Patel

(61) also were successful in isolating bacteriophage from pure cultures of *Phytomonas tumefaciens* and from artificially induced beet galls. After seventeen filtrations their agent was virulent at a dilution of 10^{-10} to 10^{-14} and was also thermostable to 80-85° C.

A bacteriophage of high potency against *Phytomonas pruni* was obtained by Anderson (2) from soil beneath infected peach trees. Typical plaques were produced though development of secondary growth was quite rapid. Anderson was not successful, however, in isolating phage from old infected peach leaves. More recently, Thornberry (70) studied this bacteriophage and estimated its size at about 11 $\mu\mu$.

A very interesting report was made by Bewley (8) in connection with tomato mosaic, a virus disease. His experiments led him to conclude that the principle causing mosaic disease of tomato is very closely allied to, if not identical with, the bacteriophage. No further work has been done in this direction but it is worthy of note that here again the very close relationship between viruses and phage has been emphasized.

Uppal (73) has isolated a race of bacteriophage specific for *Phytomonas citri*, agent of citrus canker.

One of the most comprehensive studies on bacteriophage in relation to phytopathogenic bacteria is that of Chester (21). Some of his studies in relation to the methods of isolation of phage from plants have already been mentioned. In addition, he worked out the conditions for the demonstration of bacteriophage considering the age of the bacteria used and their susceptibility, the most congenial medium for bacteriophagy, quantity of bacterial suspension to be used for testing filtrates, the amount of filtrate to be used, frequency of serial transfers, length of time of bacteria-filtrate contact, etc. Having ascertained the conditions necessary for a study of the bacteriophage, he proceeded to apply his technique to the isolation and study of the lytic agent. In studying galled and healthy stems of *Pelargonium zonale* he found that the crown-galls on these stems may contain phage. Healthy stems of galled plants may also harbor a bacteriophage, but healthy, non-infected stems yielded no lytic agent. Hence, this principle was present only in company with the disease and could move from the zone of infection to other parts of the plant; therefore, it may be of some prophylactic value in these new parts (17, 6). Only 30 per cent of

the healthy tissues of galled plants contained a bacteriophage as compared to 40 per cent of the galls. However, the distance of phage movement from the gall was only from one to several centimeters. Again, bacteriophage was present in galls on *Beta vulgaris* and in healthy parts of galled plants. In addition, he found the agent in the roots of healthy, non-galled plants, though Israilevsky (45, 46) did not. Further, bacteriophage also was present in the soil though again Israilevsky found none there. Chester believes that the agent entered beet roots from the soil, yet stems of non-galled, healthy *Pelargonium zonale* grown in the same soil contained no lytic principle. Chester suggests, however, that although the agent may have entered the roots, it was possibly too dilute to manifest itself by the time it reached the stem. It has already been pointed out that the bacteriophage of Rhizobia has been found in the roots and stems of legume plants but not in the leaves (26, 75). The same reason may hold in this case as in the above, namely, that in its upward movement, the agent becomes too dilute to manifest its activity in the leaves or stems, as the case may be. Nevertheless, the possibility that phage in soil may enter roots suggests an additional means for its prophylactic and therapeutic use.

During the past few years a considerable number of investigators have isolated bacteriophages active against a variety of bacterial plant pathogens. Massey (57) obtained a lytic principle for *Phytomonas malvaceara* (blackarm of cotton) from contaminated soil but not from clear areas. Rough colonies of this organism were not pathogenic but were destroyed permanently by the lytic agent whereas smooth colonies were pathogenic but were only temporarily affected by phage. Dufrenoy (33) isolated a lytic principle from cultures of *Phytomonas tabaca*. Thomas (69) was successful in isolating a bacteriophage against *Phytomonas stewartii* from *Coccus* bacterio-roots and the lower nodes of corn plants killed by the blood and diluted grain of a badly infected crop, and from the low on this lytic agent. of corn plants which at first showed symptom effect of serum and later recovered. Matsumoto and Okalie (58) concluded that bacteriomonas solanaceara. Wieringa and Wieker (60) is evidently in the direction teriophages from cultures of *Actinomyces farranicus* of bacteriophage. Mutsaars and applied them with Applebaum and Patterson (4) organism of potato scab, *A. scab*

little else with respect to the practical possibilities of these bacteriophages. Most recently, Biberdieva (9) obtained a bacteriophage for *Phytomonas mori*, the etiologic agent of mulberry bacteriosis.

BACTERIOPHAGE THERAPY

In Animals

Bacteriophagy *in vitro* immediately suggests bacteriophagy *in vivo* and hence the destruction of pathogenic bacteria in the body. D'Herelle undoubtedly thought of this as soon as he had demonstrated bacteriophage action against Shiga dysentery bacilli. He and very many others have attempted to use bacteriophage for therapeutic purposes in a large variety of bacterial diseases. D'Herelle (29, 31) has obtained favorable results with phage in typhoid, dysentery, coli (mainly urinary) infections, and quite favorable results in bubonic plague, fowl typhoid and in barbone. Similarly, others have obtained favorable results in the above infections though still others have failed to demonstrate any such successes (43).

Cowles and Hale (24), working with a bacteriophage highly potent against a strain of *B. anthracis*, found that it gave no protection when injected into white mice inoculated with the same strain. Flu (39) found that of 54 rats immunized three times with successive doses of 25-50-75 c. cm. of phage 44 were able to withstand 1,000 times the fatal dose of plague bacilli. Jern, Howes and Meleney (47) could establish no relationship between hemolytic and non-hemolytic properties of staphylococci and their susceptibility to bacteriophage (in cases of treatment of acute staphylococcal infections with phage). The work of Lampert, Boyce and Quency of Edge (54), however, indicates definitely that bacteriophage, etc. properly administered, is efficacious in giving prompt relief of the bacteriological cures and non-recurrence in cases of super-infection and staphylococcal infections including carbuncles, furunculosis, healthy stems of *F. f. f.*, infected wounds, and so on. Their work shows that plants may also harbor staphylococcal infections and perhaps stems yielded no lytic agent convincing been presented. The favorability in company with the disease been due to the specific immunizing fection to other parts of the plant used. . . ." Zinsser and Bayne prophylactic value in these new plants.

Jones (79) voice the same thought. They are not convinced from the available data that bacteriophage is valuable therapeutically and state that "lytic filtrates contain solutions of bacterial substance which are effective antigens for the stimulation of antibacterial immune bodies. They are, in fact, excellent vaccines." Therefore, how much of the effect in successful cases was due to bacteriophage and how much to these specific bacterial proteins? Robic (64) isolated an antiplague bacteriophage producing complete lysis of all strains of plague bacilli studied *in vitro*. But this principle had no effect in the four cases of pulmonary plague in which it was used. Still other workers have found that injections of staphylococcus bacteriophage into rabbits caused the development of hypersusceptibility to homologous staphylococcus infections (59). Similarly, Bronfenbrenner and Sulkin (16) found that bacteriophage propagated on highly invasive staphylococcus strains definitely increased the size of the lesions on rabbits when it was applied locally to the site of injection.

What are the reasons for all these conflicting results? Bronfenbrenner (15) presents a number of possible explanations for the failure of bacteriophage action in the body. Resistant or secondary cultures will not be destroyed and will develop after lysis is finished. These organisms, as has been pointed out, may be even more virulent than the original cultures. The sensitive organisms are, moreover, not subject to lysis as freely *in vivo* as *in vitro*. They multiply more slowly *in vivo* and so less lytic agent is generated. The agent itself is easily adsorbed by the cells and tissues of the body and is also rapidly eliminated from the system through the bile, urine and feces. In fact, it may be entirely gone in 24-48 hours after injection into experimental animals. Applebaum and MacNeal (3) have shown that purulent exudate exerts a marked inhibitory influence on lytic action of anti-staphylococcus bacteriophage. Undiluted citrated blood, defibrinated blood and diluted blood serum exercise an inhibitory influence on this lytic agent. Colvin (22) noted a non-specific inhibitory effect of serum and other body fluids on bacteriophagy. He concluded that bacteriophagy in the body is much modified as compared to test tube standards of lysis. The modification is evidently in the direction of lessening the sterilizing capacity of bacteriophage. Mutsaars (62) obtained similar results. Applebaum and Patterson (4)

found, however, that human bile is less inhibitory than blood or serum and suggested the possible use of bacteriophage in biliary infections. From the foregoing review one can appreciate the difficulties with which medical men are faced regarding bacteriophage therapy. Similar difficulties may well be expected in connection with phage therapy in plants. Resistant organisms may develop, plant fluids may inhibit bacteriophagy, plant cells and tissues may adsorb the lytic agent and hold it in an inactive state and the principle may leave the plant by way of the roots. Further consideration will be given to some of these aspects subsequently.

In Plants

Dufrenoy (34) is quite correct in stating that in spite of various monographs on bacteriophage and phytopathogenic bacteria, the problem has attracted very little attention from agronomists, biologists and plant pathologists. Perhaps this is due to the paucity of information concerning the action of the bacteriophage in plants. Yet the increasing number of investigations leading to the discovery of bacteriophage in diseased plants associated with the etiological agent point to a relationship which may be important perhaps with regard to therapy, in the study of immunity in plants or in relation to plant viruses.

Coons and Kotilia (23) studied the bacteriophage from the point of view of its therapeutic and prophylactic possibilities. Using fresh, moist, aseptically prepared carrot disks, they inoculated some with *Erwinia carotovora* only, others with this organism plus the homologous bacteriophage, and only where the organism was present alone did rotting occur. A similar protective action was obtained with potato slices inoculated with *Erwinia atroseptica* and the homologous lytic agent, although the results in this experiment were not quite so regular. In one case in their duplicated experiments rotting occurred where the lytic principle was present. Israilsky (45, 46) interpreted the scarcity of bacteria in crown-gall as being due to lytic action. He treated roots, stems and seeds of beets with phage before inoculating with *Phytoponas tumefaciens* and found that bacteriophage reduced very appreciably the percentage of infection, but wisely stated that a great many more experiments are needed before such utilization of bacteriophage can be unquestionably established. Kauffmann (49, 50) could not,

however, observe any prophylactic action of the lytic agent in preventing experimental gall formation, but Muncie and Patel (61) found to the contrary that cultures of *Phytomonas tumefaciens* after being subjected to bacteriophage for nine hours failed to produce infection on tomato plants. The extensive work of Chester (21) has shown that the lytic principle is present in plant tissues almost exclusively in company with the disease. This corroborates the work of the aforementioned authors that healthy plants rarely if ever yield phages active against any particular bacterial pathogen, unless, of course, the agent entered the plant from contaminated soil by way of the roots. He suggested that the greater or lesser resistance to later infection shown by daisy and rose plants with crown-galls (17) and the inhibition of reinfection of tissues several centimeters from crown-galls (5) were explainable perhaps by prophylaxis by bacteriophage diffusing out from the zone of primary infection. He claims, however, that there is a certain resistance to free movement of bacteriophage within the plant and as a result any prophylaxis manifested will be restricted. The experiments of Thomas (69) are quite encouraging as regards bacteriophage therapy. His work on the isolation of the lytic principle from corn suffering from Stewart's disease has already been discussed. He found in addition that seed corn from a badly infected crop yielded bacteriophage and this seed developed only five per cent infected plants. On treating 18 lots of commercial seed with bacteriophage and then inoculating with *Phytomonas stewarti* he obtained 1.4 per cent infection as compared to 18 per cent in the non-phage treated samples. Moreover, there was less spreading of the disease by insects from the phage-treated plants. When healthy plants received injections of phage and the organism, some showed symptoms of the disease and then recovered, others showed no symptoms whatever. Seed treatment in every case resulted in reducing the disease to a minimum. Plants attacked mildly but recovered later yielded a bacteriophage; hence, suggesting an analogy to immune reactions.

To be sure, there is much too little experimental evidence to warrant any generalized statements, but the possibility does exist that bacteriophage may be effective in reducing infection by and losses due to various bacterial plant pathogens. Reasons why bacteriophage therapy in animals may be relegated to a minor status have

already been discussed. Analogous reasons also were suggested why bacteriophage therapy may not be very successful in plants. Let us consider several of these reasons in greater detail, particularly from the point of view suggested by Jern and her associates (47). These workers stated that the minimum essentials for successful use of bacteriophage in animal diseases are: a potent lytic agent, a susceptible infecting organism, an adequate intimate contact of bacteriophage and infecting organism, and an environment conducive to the interaction of the two. They also stressed the fact that methods of application of the bacteriophage and the dosage used are very important considerations.

A potent lytic principle may be obtained with comparative ease by means of serial passages at the expense of the homologous, susceptible organism. This may then be used for plant injections, seed treatment, spraying or whatever other means may be considered feasible. But it has been noted that temperature, age, reaction and other factors affect the activity of the lytic agent even if it is assumed that the infective organism is susceptible. These factors will be considered shortly. In addition, the agent used should be preferably polyvalent; that is, it should not be specific to any one strain of the pathogen but to as many as possible. Of course, if it is polyvalent for more than one type of disease-producing bacteria, so much the better. This problem of specificity has been studied considerably in relation to plant pathogenic bacteria. Mallman and Hemstreet (55) found that the inhibitory substance they isolated from rotted cabbage inhibited not only the cabbage rot organisms but also *Bacillus speckerman* and *Erwinia carotovora*, although after a number of serial passages this inhibitory power was lost for all except the cabbage rot organisms. Coons and Kotilia (23) isolated a bacteriophage from carrots active on *Phytomonas tumefaciens*, *Erwinia carotovora* and *Erwinia atroseptica*. Israilsky (45), as has been pointed out, obtained only two susceptible *Phytomonas tumefaciens* strains, while Hitchner (44) isolated a lytic agent from the root nodules of red clover active against only one strain of clover organisms from the same nodules. Biberdieva (9) has obtained so specific a lytic principle that he suggested its possible utilization in identification work. Many others in medical bacteriology (65, 56) have begun to use bacteriophage for species differentiation. In addition, Muncie and

Patel (61) found after testing 20 strains of *Phytomonas tumefaciens* and eight other bacterial plant pathogens that their bacteriophage was specific only for the strain from which it was isolated. Similarly, Matsumoto and Okalie (58), having isolated a bacteriophage for *Phytomonas solanaceara*, found it to be inactive against 13 other bacterial species tested. It is evident that most of the phytopathogenic bacteria for which a bacteriophage has been demonstrated are heterogeneous species, capable of producing both susceptible and resistant strains. Laird (53) and others (26, 75) have shown that the Rhizobia are similarly heterogeneous. It would be highly desirable to so activate the agent that it would dissolve all the strains of a particular species of bacteria, but this has not yet been accomplished to any extent, nor is it a simple matter, perhaps even impossible although d'Herelle (29) and others, including the writer, have obtained such complete dissolution that the medium remained to all intents and purposes perfectly sterile.

This question of specificity brings us to the very important problem of resistance to bacteriophagy. Obviously, if the lytic agent dissolves only some of the organisms produced by a certain pathogenic species, the others may continue to develop and produce the disease. Some authors claim, too, that resistant organisms are more virulent than the original cultures (12, 35), others that the resistant forms are less virulent (13, 38). We have seen that Bronfenbrenner and Sulkin (16) obtained deleterious effects in the local application of *Staphylococcus* bacteriophage. Yet Burnet (19) claims that the failure of bacteriophage to induce therapeutic results cannot be attributed to the appearance of resistant bacteria. The frequently observed fact that bacteria resistant to one strain of bacteriophage may be susceptible to lysis by another strain (14) may support Burnet in that several races or strains of bacteriophage may be present in the plant, either entering from the soil or with the infecting organisms. Furthermore, Wollman (78) claims that bacteriophage can act on both living and dead resistant organisms provided living, susceptible strains of the homologous organism are present. Twort (15) came to similar conclusions though Bronfenbrenner (15) showed that this was not the case and stated in addition that "so long as live bacteria are present, increase in bacteriophage could not be attributed directly to assimilation by phage of dead bacteria." There still remains the possibility,

however, that such "secondary lysis," as Wollman (78) called it, may occur with certain bacterial species. Inherent or acquired resistance to bacteriophage is then a serious consideration in phage therapy. In addition, certain bacterial species produce slime which would, of course, provide a mechanical barrier against the lytic principle. The suggestion of certain workers (41) that slime production is a specific protective action on the part of bacteria is open to question since slime producers are indiscriminately resistant to all lytic agents (51), but if the slime is removed, the clean bacteria are susceptible (67). Laird (53) has shown that growing Rhizobia in a mannite-free, yeast-water medium reduces slime production markedly and enhances bacteriophage action. Hence, the protective action of slime would not obtain if conditions in plants were such as to preclude slime production. Of the phytopathogenic bacteria for which bacteriophages have been isolated, *Phytomonas solanaceara*, *Phytomonas tumefaciens*, *Erwinia atroseptica* and *Phytomonas citri* produce slime (37) and even then only under certain conditions. Again, Berge, Riker and Baldwin (7) correlated pathogenicity and viscosity in cultures of *Phytomonas tumefaciens* (viscosity, of course, being related to slime production). They found that pathogenic strains were less viscous than non-pathogenic strains; consequently, pathogenic forms would be protected less by viscous slime and would be more susceptible to bacteriophage action. From these considerations it is evident that slime production need not offer too serious an obstacle to lysis.

Spore producers may resist bacteriophagy but numerous investigators have isolated lytic agents for *Clostridium tetani*, *Bacillus anthracis* and other spore producing organisms (24, 25, 32). Moreover, it may be justifiably assumed that in active disease relatively few if any spores are present and so only the vegetative forms will be exposed to the lytic principle. Nevertheless, it would be interesting to know if the bacteriophage of a certain spore-forming organism can lyse a spore suspension of that same organism.

The second minimum requirement for effective phage therapy deals with the necessity for an intimate bacteriophage-bacterium contact. Treatment of seeds with bacteriophage preparations prior to planting into soil known from previous experience to harbor the homologous infecting organism is undoubtedly one of the most practical prophylactic measures. But how long will the bacterio-

phage remain in the soil? Demolon and Dunez (26) have found that when soils are desiccated, the agent disappears fairly rapidly. Otherwise it may persist in the soil for a long time since it is fairly thermostable, resists cold temperatures and is on the whole fairly resistant to various harmful agents. As long as conditions in the soil are suitable for bacterial life, the agent will remain in the soil. Now if homologous organisms are present in the soil, the bacteriophage will regenerate at their expense and as a result will persist in the soil for even a longer time. Hence, it may be present in sufficient strength to tide the plants over the more susceptible period of their growth. Of course, it can and will be diluted by rain and soil water, but it has been shown that the agent is active even at extremely high dilutions. Lampert *et al.* (54) have shown that frequent applications of bacteriophage are necessary in the treatment of skin infections, abscesses, etc. Frequent spraying with the bacteriophage would then be the logical step unless the potency of the agent could be greatly enhanced, but this method is not practical especially with crops such as corn grown on a large scale, although with crops grown on a smaller scale, such a method may be feasible. Spraying will add bacteriophage not only to the soil but also to the plant (internally by diffusion from the soil into root and stem and externally on any diseased parts of the tissues exposed by the action of the pathogen). In irrigated soils the problem of applying bacteriophage is much less complicated as it can be added to the water. Where bacterial diseases of trees are concerned, direct injection of the lytic agent would probably be the most effective method, although spraying also could be done.

These considerations are, of course, more or less speculative. One must bear in mind that large quantities of bacteriophage would be necessary. Economically the cost of its production as compared to the cost of bactericidal preparations on the market today might very well be prohibitive, yet there would be this advantage that bacteriophage filtrates would leave no residue in the soil which on accumulation might become harmful to plant growth, as for example, is the case with lead arsenate sprays (74). It must also be recalled that Bewley's work (8), in which the results pointed to a definite similarity between bacteriophage and mosaic virus of tomato, is as yet not definitely refuted. From this point of view the treatment of crops, especially tomatoes with bacteriophage,

would be analogous to treatment with a virus, a practise hardly recommendable.

The final condition for optimal activity of bacteriophage as a therapeutic agent is a suitable environment for the interaction of the lytic agent and the infecting organism. Some of the factors for optimum bacteriophagy have been discussed previously. The chief consideration is whether or not the pathogenic organism is developing more or less normally in the plant or in the soil. If it is (as it should be if it is to cause infection), then bacteriophage will be able to act on it because the lytic agent is active on living, multiplying organisms and because the most congenial medium for bacteriophagy is one which is optimum for the growth of the homologous organism. If the organism is not multiplying actively, bacteriophagy is inhibited to a certain extent at least, but the agent can and usually does remain in close association with the pathogen.

An interesting question may have arisen as to the possible relationship between the development of immunity in plants and the bacteriophage. The question is a difficult one, for the study of immunity in plants is still in its infancy. If the bacteriophage is itself a virus disease of bacteria as d'Herelle claims (29), a stage in the life cycle of bacteria as Hadley (42, 43) suggests or an enzyme produced by bacteria as many workers contend (43), it cannot be considered in connection with antibodies and immune reactions. If, however, it is produced by the plant or animal cells or tissues in response to the parasitic stimulus, it may be related in one way or another to antibodies. There are a number of workers (29, 43) who believe the bacteriophage is an inanimate entity foreign to the bacterial cell, although the majority by far believe that it is derived from the cell, so that the possibility that the lytic agent may be related to antibodies is more or less obviated. The injection of bacteriophage into experimental animals results in the formation of anti-bacteriophage antibodies. A similar phenomenon may occur in plants, but as far as the author is aware no bacteriophage has been produced as a result of the injection of phage-pure organisms into plants or animals.

Does the bacteriophage ever exert harmful effects? Obviously if, as a result of its action on homologous organisms, resistant strains develop which are more virulent than the original cultures, its use could hardly be considered beneficial. Again, Bewley's

claim that bacteriophage and tomato virus are one and the same would suggest that the lytic agent is more deleterious than useful. Whitehead and Cox (76) have found definite indications that the loss of activity by cheese "starter" cultures was due to the action of bacteriophage on the lactic streptococci in the starter. Bronfenbrenner and Sulkin (16) and others have shown that certain bacteriophage treatments have done more harm than good. More closely related to plants, however, are the studies of bacteriophage and its possible relation to "fatigue" of soils on which alfalfa has grown for a number of years. Demolon and Dunez (26) concluded from their experiments that Rhizobia bacteriophage in soil brings about the destruction of the nodule bacteria in the soil, thus interfering with normal symbiosis and resulting in decreased yields of alfalfa. Vandecaveye and Katzenelson (75) have similarly isolated Rhizobia bacteriophage from alfalfa fields but did not commit themselves to any definite statement regarding the harmful effects of bacteriophage other than to state that the possibility exists that the lytic agent is responsible in part at least for reduced alfalfa yields. Others (6, 20) obtained results similar to the above but they did not come to any definite conclusions. They did suggest, however, that bacteriophage may be a factor in bringing about lower yields of alfalfa, but is very probably not the only one.

Perhaps the chief objection to the possibility that bacteriophage interferes with normal symbiosis is that the Rhizobia are heterogeneous with respect to the bacteriophage; that is, they can produce both susceptible and resistant strains. Now if the lytic agent destroys the susceptible strain, the resistant ones can still multiply and produce nodules, and it has been shown (53, 1) that resistant and susceptible strains are alike in their ability to produce nodules on leguminous plants. Of course, the total number of organisms capable of nodule production is reduced by the bacteriophage and if the principle becomes potent enough, it may even destroy some of the resistant forms. Razumovskaja (63) has shown that the addition of bacteriophage to soil caused a decrease in the development of nodule bacteria during the first few days; later, resistant forms developed fairly well. As a result there will be fewer organisms for nodule production, a condition which may manifest itself by decreased yields of legumes.

Here, then, are interesting possibilities and perhaps important ones in relation to bacteriophage in plants. In the case of the Rhizobia the ultimate effect of bacteriophage activity may be harmful since the Rhizobia are themselves beneficial. In the case of phytopathogenic bacteria, recognizedly harmful to plants, the bacteriophage may be beneficial. As in so many other fields of science, the future holds the solution to this problem.

ACKNOWLEDGMENT

The author wishes to extend his appreciation to Dr. F. D. Heald of the Department of Plant Pathology for his critical reading of the manuscript and for his many valuable suggestions.

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RECENT ADVANCES IN THE PHYSIOLOGY OF LATEX

LAURENCE S. MOYER

Department of Botany, University of Minnesota

Aside from the commercial importance of latex as a source of rubber, gutta percha, opium, papain, and even chewing gum, its widespread occurrence in so many families of plants has aroused the curiosity of many botanists. For not only *Hevea*, *Palaquium*, *Papaver* and *Achras* contain latex, but also such remotely related genera and species as *Lactaria* and *Russula*, *Acer platanoides*, *Asclepias*, *Lactuca*, *Taraxacum*, *Ficus* and many others. The production of latex may assume no inconsiderable part in the metabolism of the plant; for instance, it has been noted by Bobiloff (23) that there are trees of *Hevea* which yield 80 kg. (175 lbs.) of rubber over a ten-year period. The literature of this subject is scattered through many rather inaccessible journals; the present paper will attempt to select some of the most important recent work from the standpoint of plant physiology. Several helpful reviews of the subject are found in the works of Bobiloff (23), Memmler (60), Frey-Wyssling (41), and Hauser (48),

OCCURRENCE AND COMPOSITION OF LATEX

Latex vessels are living cells. Nuclei have been found *in situ* and in the exuding latex by a number of investigators after the initial discovery by Treub (95), Scott (87), and Schmidt (85). Calvert (30) reported the presence of protoplasm and Calvert and Boodle (31) noted the coenocytic nature of these cells. In addition to confirming the presence of nuclei, Potter (79) found leucoplasts in laticiferous vessels of *Euphorbia splendens*.

The peculiar structure of the nuclei in latex vessels was first reported for species of *Musa* and certain aroids by Molisch (61) who refers to them as "Blasenkerne." These nuclei appeared to be lying in a vacuole. This he interpreted as really caused by a vacuole in the nucleus itself. Bobiloff (19), working on *Hevea*, has shown that unusual nuclei are also characteristic of the laticiferous vessels of this plant. The outer part of the nucleus appears less filled with chromatic material than the rest and seems to be sepa-

leaves, have suggested the possibility of the latex system as a storage or transporting organ. These anatomical peculiarities, together with the general anatomy of latex vessels and cells, have been well reviewed by Haberlandt (43), Bobiloff (23), Frey-Wyssling (41), Memmler (60), and Böttcher (25), to whom the reader is referred. Ultimate proof, however, demanded experiments. The early experimental workers investigated the effects of ringing and starvation on the percentage of starch and other substances. The older literature has been reviewed by Molisch (61). Thus it was found by Groom (42) that when leaves of *Euphorbia peplus* were placed in water and exposed to light, starch was noted in the cells but little could be seen in the latex vessels. Starch did not leave the vessels when the leaves were placed in the dark, in confirmation of the results of Schimper (84). On the other hand, Biffen (12) found the sugar in latex increased at the end of a photosynthetic period (in *E. peplus* and *E. pulcherrima*) and that, on darkening, the percentage of sugar diminished markedly. Kniep (54) concluded, in agreement with Groom, that the starch of latex vessels was not a typical reserve for the other tissues, for, when plants were kept in the dark until etiolated, a loss of starch was noted in all tissues except the laticiferous system. The same results were obtained by Bruschi (27) who also found a decrease in fats in the latex vessels under unfavorable conditions. Bernard (10) reports a starch decrease on etiolation. Orken (77) and Bobiloff (23), however, were unable to confirm this. Some of these workers were content to investigate the amount of starch present in exuding latex but, as it has been pointed out by Frey-Wyssling (41), this is scarcely sufficient, for the bulk of the starch comes out only under pressure.

Seasonal variations in dry weight or turbidity of the latex of various plants have been reported by Jumelle (50), Zimmermann (103), Tobler (94), Rae (80) and Kiselev and Kuz'mina (52). None of these techniques could be used to decide whether a change in dry matter or a change in water content had taken place.

More positive evidence for environmental effects has been presented by Roeben (81) who found that the dry weight and specific conductivity of latex decreased in *Euphorbia lathyris* grown in a dry atmosphere. Under these conditions, there was no decrease in the leaf dry weight. In agreement with investigators who have reported a decreased milkiness in plants kept in the dark, Roeben

found a lowered dry weight of latex. The lowered conductance rises again if plants are taken from dry air to humid surroundings. These results were interpreted as a consequence of decreased photosynthesis, stomatal closure, and the movement of organic substances and electrolytes to neighboring cells. For details as to the effects of growing plants in salt solutions, *etc.*, the original paper should be consulted. Bobiloff (17, 23) has placed *Hevea* seedlings in salt solutions and finds that the transpiration rate does not remain normal, as might be expected if the latex ducts were serving as water reservoirs, but decreases immediately. He has also presented evidence (20) to show that the respiration of *Hevea* plants is normal and not conditioned by the presence of latex. Upon placing the plants under anaerobic conditions, no decrease in rubber content was noted.

Proof of the utilization of rubber required the analysis of the total rubber content of whole plants. Such estimations by Bobiloff (23) have shown no decrease in rubber content (as percentage of seedling dry weight) of rapidly growing *Hevea* seedlings kept in the dark, after removal of the seed, until the starch reserve was exhausted (after two months). The mass of terpenes stayed constant or increased slightly, apparently in consequence of the weight decrease of the plant through respiration. In another experiment, two groups of seeds were germinated, one group in the dark and the other in the light. Thirty-five days later, although those in the dark had only a slight amount of food material, both groups had the same rubber content (.69 per cent). Bobiloff also points out that the latex in the leaves of *Hevea* is not depleted before abscission but stays about the same, in common with other excreted substances. In general, he concludes that latex in *Hevea* is an excretion. It has been claimed by Onken (76) that the vessels serve as storehouses in which insoluble lime is held. Bobiloff, however, states that although leaves of *Hevea* contain numerous druses of calcium oxalate, no crystals can be found in its latex. The cotyledons of many members of the Moraceae (*Artocarpus*, *Castilloa*, *etc.*) contain rich masses of latex, yet Bobiloff found no decrease in rubber content up to the full emptying of the seed.

Opposite results were obtained by Spence and McCallum (90) working with *Parthenium argentatum*, the guayule. As shown by Lloyd (56, 58), this plant carries its latex colloidally dispersed in

the vacuoles of a layer of cells overlying the cambium and in the living cells of all permanent tissue, but does not possess a special system of laticiferous ducts. There is no rubber in the leaves or other temporary tissues. The root contains as high a percentage as the stem and represents 45 per cent of the total weight. Spence and McCallum present evidence that rubber from guayule is easily broken down by bacteria or enzymes and suggest that this could be carried out by the enzymes of the plant itself, possibly transforming it to pentoses, as suggested by Harries (76).

In their first demonstration of utilization of latex by the plant, fifty practically identical two-year old plants were divided into two lots. The tops were trimmed off and the one lot was set out in beds. The other lot, the control, was analysed at once. After three months, the plants which had been growing were taken up; the new growth was separated by hand from the old and analysed separately. The results showed a loss of 10 per cent of the rubber which was present in the controls. The new growth had produced a dry weight equal to that already present in the old growth (which remained unchanged) but the rubber content in the new was only .045 gm., a negligible amount as compared with the 2.35 gms. in the controls. The total rubber per plant had dropped from 2.35 gms. to 2.10 gms. The addition of 19 gms. of dry weight per plant was accompanied by a decrease in rubber content during the period of rapid growth. In repeating this experiment, using washed sand instead of earth to eliminate increases from the soil, it was found that 28 per cent of the rubber in the plants at the start was gone at the end of two months. Virtually no rubber was formed by the new growth. No rubber was found in the leaves or floral stalks at any time. The chief difference between this and the preceding experiment was in the loss of dry weight by the old trunks and roots on being grown in sand.

To investigate the possibility that new leafage and growth after dormancy was brought about at the expense of energy from rubber, the authors defoliated half of a lot of 48 four year old plants, reasoning that defoliation should accentuate the demands on the plant's rubber supply. Controls and defoliated plants were grown for a year and sampled at monthly intervals. At the start, the rubber content of the defoliated plants was within 5 per cent of the controls (undefoliated). During the growing season, no rubber

was produced or lost by the controls but, after August, when the plants began to dry up, they laid down 22.5 gms. of rubber per plant, an increase of 32 per cent over the initial amount in May. The defoliated plants, however, lost 8 per cent of their total weight per plant and 10 per cent of their rubber content in breaking dormancy. In all, the defoliated plants lost over 10 gms. of rubber per plant, only half of which was replaced.

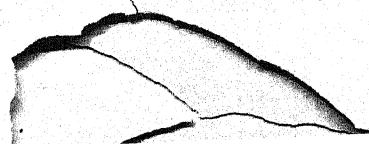
As a further experiment, 25 plants were fully defoliated, 25 half-defoliated, and 25 grown as controls. All plants were four years old and as alike as possible. As before, no rubber was ever found in the leaves. In the controls, the rubber content remained fairly constant from May—during the growing season—then increased steadily after the moisture content dropped between August and January. After this it decreased again to a value near the initial level. Meanwhile, the defoliated plants lost steadily until August when the amount remained nearly constant until December. After this a steady decline took place to a much lower level than at the start; in fact, 30 per cent of the original rubber disappeared. The results from the half-defoliated plants lay midway between these two sets, indicating that defoliation makes the plant draw on its rubber.

Spence and McCallum then experimented with three rows of dormant three year old plants in August which had not been watered for a year. Their leaves had fallen and, as shown by the previous work, they were in the stage of rubber formation. After controls were analysed, water was added to the rest. The irrigated plants blossomed and grew, into October, during a period when they were normally dormant. Analyses at the middle of August, October and January showed a falling off in rubber content; when the plants were normally gaining rubber, 18 per cent disappeared.

Although this utilization of latex has not been demonstrated as yet in *Hevea*, it is possible that such methods as are here described will be applied with success, or else, as the experiments of Bobilioff seem to indicate, *Hevea* will be found to be different in its metabolism from *Parthenium*.

FLOW OF LATEX AFTER TAPPING

The physiology of the tapping process, both as to the extent of the area involved and the cause of the flow, has been investigated



by several authors, among them Arisz (5, 6, 7), Bobiloff (13, 14), Vischer (99, 100) and Zimmermann (103). The whole subject has been exhaustively investigated by Frey-Wyssling (39, 40). In agreement with Arisz and Zimmermann, he was able to demonstrate that during its outflow the latex becomes thinner in consistency. By collecting the yield in fractions, it could be shown that, at the start, the latex had 39.1 per cent dry matter. On tapping, this value decreased to a minimum at 33.2 per cent, after which it rose a little. This might be explained as (1) a dilution from the sieve tubes, (2) an abnormal dryness of the latex near the site of cutting, or (3) a real dilution due to internal factors in the vessel. He points out that other workers find little if any flow of sap from phloem and that such a flow would occur in the first fraction where it would cause a lowering of the initial concentration. To test the second possibility, he drew samples with a bark tester in the area near the cut. He found no systematic increase in water content as the distance from the cut was increased and concluded that a real dilution was taking place. By analysis of the curve of water content *vs.* volume of latex, it could be shown, assuming an initial concentration of 39.1 per cent, that 14.5 per cent of water, based on undiluted latex, was added. This figure is equivalent to 23.8 per cent of the original volume of water present. No effect of weather conditions could be noted. On tapping a strip of isolated bark, a dilution reaction was also noted, amounting in this case to two-thirds of the original serum.

To show the possible effect of root pressure on the dilution reaction, a tree was felled and all cut surfaces were covered with paraffin. On tapping, the tree continued to yield as an intact tree for a period of five days and evinced the dilution reaction each day. It follows that the root has little influence on the flow and shows that some mechanism must be present in the bark to regulate automatically the flow of latex during tapping.

Frey-Wyssling always found, on reopening the cut, that the latex had regained a higher solid content as compared with the maximal dilution of the preceding tapping, even after only a few hours' rest. This "rejuvenation" may be made to occur up to nine times a day.

On tapping a tree, the latex flows rapidly at first and then decreases in rate. By applying the law of Poiseuille,

$$V = \frac{\pi r^4 P n t}{8 \bar{\eta} L} \quad (1)$$

(where V is the volume exuded in the time t , through n tubes of average radius \bar{r} , and length L , under a pressure P ; $\bar{\eta}$ is the average viscosity), Frey-Wyssling could derive the hyperbolic expression,

$$V = m \left(1 - \frac{1}{kt + 1} \right), \quad (2)$$

to describe the volume of flow with time. Here m is the maximal volume and k is a measure of the original slope and possesses the dimensions of t^{-1} .

Both this and the differentiated expression for rate of flow,

$$Q^* = \frac{dV}{dt} = \frac{k}{kt + 1} (m - V), \quad (3)$$

which has the shape of a die-away curve, could be fitted to his data very well, except for the initial value of Q^* . The initial value observed, Q_0 , was always significantly higher than the initial calculated value, Q_0^* .

The volume of the vessels must change during the flow or the latex would not leave them. This initial higher rate, followed by a rapid decline in the observed rate of flow, Q , to that demanded by equation 3, is probably due to a change in r , by the law of Poiseuille; so that to find the initial value for the radius, the following proportion holds:

$$Q_0 : Q_0^* = \frac{R^4}{\eta_0} : \frac{\bar{r}^4}{\bar{\eta}}, \quad (4)$$

where Q_0 equals the observed initial value of Q ; Q_0^* the initial value ($= km$) calculated from equation 3; R the radius at the start; the other variables are as before.

Equations 1, 2, and 3 rest on a *mean* viscosity. A determination of the initial viscosity, η_0 , would permit estimation of these changes in the dimensions of the vessels during the flow. Frey-Wyssling found that the data of de Vries (101) for the relative viscosity of latex as a function of concentration could be fitted closely by an exponential curve, enabling an extrapolation to higher latex concentrations.

Data for a series of 27 tappings on the same tree, over a three-month period, were used by Frey-Wyssling to test this hypothesis of a change in the radius after tapping. The various experimental tapping curves gave greatly divergent values of Q_0^* and Q_0 . It was also found that $\bar{\eta}$ decreased from day to day during the experiments to about 25 per cent of its value at the beginning of the series. η_0 changed also. Yet when R was calculated by equation 4, it was found that the ratio of \bar{r} to R was constant, i.e., $\bar{r} = .80R$, with a standard error of only 1.2 per cent of the mean (calculated by the writer). This indicates that the radius decreases rapidly to less than 80 per cent of its initial size.

It can be shown that the initial rate of outflow is 3.2 cm./min. and may even reach 5-6 cm./min. The velocity distribution through the tube has a parabolic shape similar to that encountered in electrophoresis cells (73); motion near the wall is negligible so that there is no force tending to push the protoplasm from the cell with the latex. This is important, for otherwise latex could not be regenerated by the vessel once it was cut.

By the use of equation 1, Frey-Wyssling was able to estimate the maximal distance from which the latex moves to the cut. This was found to be about one meter. Other calculations based on the measured dimensions of the vessels and the ease of flow around the medullary rays gave essentially the same result. It was also found that resistance met by latex when flowing horizontally is about 9 times as great as when moving vertically. These figures for the maximal distance affected are in good agreement with experiments by Vischer (99), who measured the distance at which a flow of latex was no longer affected by a cut above, and Bobiloff (16) who determined the area in which yellow latex is changed into white during tapping.

Consideration of the forces involved has shown that the turgor pressure of these vessels amounts to several atmospheres. Indeed, Bangham (8) reports that piercing a mature fruit stalk of *Cryptostegia grandifolia* near its base, after a shower, produces a spurt of latex for 2-3 seconds reaching 3.5 feet. This was also noted in *Hevea* in the early morning or soon after a shower. Changes in turgor of *Achras zapota* have been investigated by Karling (51) using MacDougal's dendrograph; he also finds maximal turgor in early morning.

Frey-Wyssling has discussed in detail the forces effective in moving the latex from the cut vessels. On cutting the vessels, the turgor decreases locally to zero. At a distance L from the cut, the original turgor pressure, T , is retained (100). In the experiments of Bobilioff (18), it was noted that on cutting isolated vessels of *Carica*, the latex was emitted even though there was no pressure on the wall from surrounding cells. In the natural state in the plant, the turgor is probably made up of the wall pressure, W , and the outer pressure, A , from surrounding cells, so that

$$T = W + A. \quad (5)$$

As pointed out by Arisz (7), the equation of osmosis,

$$S = O - T, \quad (6)$$

where S is the suction tension (Saugkraft) and O the osmotic pressure, may be applied to the latex vessel. Hence, with a fall in T , an increase in S must take place. This increase in S is inversely proportional to the distance from the cut, being greatest where T is zero, i.e., at the site of cutting, and less in the main body of the tube.

The change in turgor, amounting to several atmospheres, sets up a flow of latex toward the low pressure area similar to the extrusion of water from a medicine dropper. The rate of flow, Q , will be proportional to the gradient, dT/dL , so that, at the start, when the decrease in pressure is distributed over a short distance, Q will be high but will steadily decrease as the length involved increases. This explains the die-away character of the rate of flow curves (equation 3) (aside from the change in the radius).

The extremely high initial value of S set up near the cut will cause water to be sucked from adjacent intact cells (whose value of S is that which obtained before cutting the vessel) and cause a sudden strong dilution of the latex exuding from the plant. As time goes on, the length affected becomes greater and the gradient less, so that the subsequent dilution will proceed more gradually. The neighboring cells which have given up their water have their suction tension raised by the resulting drop in turgor, so that water is moved in a steady stream from all nearby cells (103). Hence, the course of the dilution reaction is completely accounted for by

osmotic considerations. This dilution lowers the viscosity so that the flow is facilitated.

As soon as the latex coagulates at the cut, the nearby cells which have lost water assume a higher suction tension, for now T in the vessel becomes effective once more and, furthermore, the osmotic pressure of the latex has been lowered by the dilution. Under these new relationships, water moves through the walls of the vessel back to the cells until $S_{cells} = S_{vessels}$, as it did before tapping, and the concentration of the latex becomes more nearly what it had been. Frey-Wyssling then discusses these laws of latex flow as a special case in the general phenomena of bleeding in plants.

SPECIFICITY OF LATEX PARTICLES

Latex particles, like other colloidal particles, carry an electric charge which produces an electrokinetic potential at their surfaces (1, 2, 71). The magnitude of this potential may be determined by measuring the rates of migration of individual particles in an electric field. Abramson (1) and Moyer and Abramson (73) have shown that the behavior of dissolved proteins may be investigated by suspending quartz or similar particles in the solutions, whereupon the particles adsorb the protein and move with the same mobility as the protein in its dissolved state. The whole field of electrokinetic phenomena has been discussed in a book by Abramson (1), which covers the literature to 1934. For a list of the more recent papers, the reader is referred to Moyer (72). Later developments in theory and their applications to biology and medicine have been reviewed by Abramson and Moyer (2, 3), Bull and Moyer (29), Moyer (71) and Moyer and Bull (74).

Due to its amphoteric nature, a protein surface is markedly influenced by changes in the hydrogen ion activity. Its initial net negative charge at high pH values decreases as the pH is lowered until at the isoelectric point the particle does not move, *i.e.*, the net charge on the protein is zero, and then, as the pH is lowered still further, the net charge density becomes increasingly positive.

It is generally agreed that the latex particle surface is composed of adsorbed material taken up from substances in the vacuole of the living laticiferous cell. Hence these physico-chemical methods could be applied to latex to investigate the composition of the sap vacuole as reflected by the surface of the latex particle. This

adsorbed coating gives stability to the latex particle; when acetic or other acids are added during the processing of latex, the resulting coagulation is caused by the discharge of the particles at their isoelectric point (36, 45).

The oxidases in *Hevea* latex have been investigated by Bobilioff (24) who found that by their color reactions to calcium salts he was able to distinguish between clones but, until recently, no other chemical classification, using the other constituents of latex, had been made.

It has been shown by Moyer (64), using techniques described in detail elsewhere (1, 3, 4, 62, 69), that the isoelectric points of latex particles from various species of *Euphorbia* and *Asclepias* were constant within .1 pH unit when these latices were measured in m/50 acetate buffers.² This constancy depended, within broad limits, only upon the species and not upon environmental differences. Electrophoretic mobility-pH curves, determined for several species obtained from different sources, were identical within the limits of error and could be used, therefore, to characterize a species.

The electrophoretic mobility-pH curves from over twenty species of *Euphorbia* exhibited marked differences from species to species (62). Curves possessing similar shapes and isoelectric points were grouped, whereupon it was noted that these groups coincided, very nearly, with the classification established by systematists. A marked correlation appeared to exist between similarities in geographical distribution and curve shape. For further details the original communication should be consulted.

An apparent exception was noted in the section Poinsettia. Although the poinsettias agree for the most part, *E. heterophylla* was markedly different from the others in curve shape and in the posi-

² The conclusions of Daniel, Freundlich and Söllner (Trans. Faraday Soc. 32: 1579. 1936) and Kemp and Twiss (*Ibid.* 32: 890. 1936) as to the effect of dilution on the electric mobility of the latex, based as they are on experiments with *preserved* latex, would not seem to apply to our investigations. Experiments performed at different dilutions have never shown any differences as long as fresh latex is used. Inert particles coated with egg albumin first show the effect of dilution by an increased variation between electric mobilities of individual particles, when the protein concentration is reduced below a certain point, indicating incomplete coating (Moyer, L. S., *Jour. Phys. Chem.*, in press). Under our conditions, latex particles varied no more than has been customarily observed with other surfaces. This indicates that the surface coatings of particles from fresh latex are tightly adsorbed. The customary odor of putrefaction of "preserved" *Hevea* latex probably indicates a partial transformation of the proteins to poorly adsorbed split products.

tion of its isoelectric point. A count of the chromosomes of these species (63) revealed that *E. heterophylla* was tetraploid with 56 somatic chromosomes whereas the rest of the members investigated from this group had a diploid complement of 28 chromosomes. In addition, it has been shown (70) that latex particles from the genus *Asclepias* exhibit this specificity in their electrophoretic behavior. The curves of the species investigated were so similar in shape that each appeared to form part of a single hypothetical curve depending on the position of its isoelectric point. Although *A. syriaca* has 24 chromosomes ($2x$) instead of the complement of 22 shown by the rest of the species investigated, no marked difference was noted in its curve shape, except possibly in more marked changes in slope.

These results emphasize differences and similarities in the latex particle surfaces. The positions of the isoelectric points on the pH scale together with the available chemical data strongly suggest that most of the surfaces are at least partly composed of proteins. Some species probably have particles coated with a single protein, but in others the system is undoubtedly complex. In certain cases it is doubtful if proteins play much of a role, for the isoelectric points of many species of *Euphorbia* lie near pH 3, a value rarely given by proteins. Latex from most of these species gave negative color reactions for proteins. Most authorities agree that latex contains sterols (9, 11, 28, 32, 38, 45, 53, 75, 102). An investigation of the electrokinetic properties of carefully purified cholesterol (65, 67) and ergosterol (66) showed that these two sterols were identical in behavior, within the limits of error, becoming isoelectric at pH 3.2. The shape of the mobility-pH curve was markedly similar to curves yielded by latex from certain species of *Euphorbia* (62).

Beyond this, electrophoresis has given little information concerning the other factor governing stability—the hydration. A more direct approach to this problem was afforded by the investigation (68) of the wetting properties of latex at an oil-water interface, using the Mudd interfacial technique. This consists in lowering a cover slip on a drop of a suitable oil and a drop of the aqueous latex suspension so that the two drops meet at an interface; the behavior of the latex particles in this moving interface are observed with the dark-field microscope. Particles which are not protected from being wetted by the oil pass into the interface with ease, whereas those with a coat of protein or other hydrophilic substance resist

passage. Latex from species of *Asclepias*, *Ficus*, *Euphorbia* and *Musa* showed divergent behavior in this respect. *Asclepias*, *Ficus* and *Musa* particles strongly resisted wetting, as did those from *Euphorbia* species with high isoelectric points and smooth protein-like electrophoresis curves. On the other hand, latex particles from species of *Euphorbia* with low isoelectric points showed preferential wetting by the oil, in agreement with their possible lipoid surfaces.

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THE BOTANICAL REVIEW

VOL. III

NOVEMBER, 1937

No. 11

CHIMAERAS: A SUMMARY AND SOME SPECIAL ASPECTS

W. NEILSON JONES

Hildred Carlile Professor, Bedford College, University of London

In an article such as the present it is obviously impossible to attempt to describe and discuss adequately even the better known chimaeras. Fortunately, no such feat of compression is called for since the subject has been reviewed in some detail recently by the writer in an easily available text (31) and by other authors (8, 25, 40). It would seem better to use the space now available for summarising what has been learned regarding chimaeras, especially from more recent investigations, and for discussing their significance in so far as they throw light on particular biological problems.

A plant chimaera may be defined broadly as a plant, or part thereof, which is not genetically uniform throughout. Thus, it should be possible to obtain from a chimaera more than one type of plant by vegetative propagation from the genetically unlike tissues composing it. This theoretical possibility cannot always be realised, however, owing to the practical difficulties associated with vegetative propagation from some tissues, and proof of the composite nature of the plant must be derived from other sources.

TYPES OF CHIMAERAS

A primary question in the case of any chimaera is how the genetically unlike regions are disposed. A number of different arrangements occur, of which the stability during growth varies:

(A) One or more branches differ throughout genetically from the rest of the plant. Such a result is obtained when one variety of plant is grafted on a stock of some other variety, or as a consequence of the spontaneous rearrangement of the components of one of the types of chimaeras to be described later. It is conceivable that a similar result is sometimes attained in nature as a consequence

of all the cells composing a meristem simultaneously undergoing a similar vegetative mutation, although somatic mutations yield most frequently the mericinal type of chimaera.

It is unlikely that a composite structure of this kind will be maintained for long except under cultivation, where one type of branch can be prevented by pruning from growing at the expense of the other and overpowering it.

(B) The sectorial type of chimaera in which the growing point of the branch is composed of two components united side by side laterally. The tissues that become differentiated from such a meristem as it grows forward will have the same disposition, the tissues on one side of the stem differing genetically from those on the other. Such an arrangement may be comparatively stable, so far as the main branch is concerned, but is not so in respect to lateral branches, the character of any branch depending on the position in which the bud producing it arose on the main stem. Only those buds arising at the junction between the two components will have a chimaeral structure; the rest, forming the majority, will be composed entirely of one or the other component (Fig. 1).

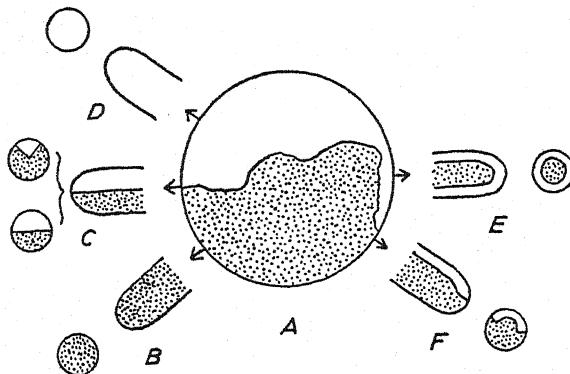


FIG. 1. Diagram to illustrate that branches from a sectorial chimaera (shown in cross section at A) may be pure for one or other component (B and D) or possess a sectorial (C), pericinal (E), or mericinal (F) structure depending on the position of origin.*

* Reproduced from (31) with permission of Messrs. Methuen and Co.

The chimaeral branches that do arise will have a sectorial structure like the main branch when the line of junction between the two components is radial; when, however, the line of junction is strongly tangential near the surface, the chimaeral branch may have a peri-

clinal structure, that is, one component may form a "skin" of uniform thickness over a "core" of the other, the number of cell layers involved in the "skin" differing in individual cases and depending on the thickness of the overlap.

(C) This periclinal type of chimaera is by far the most stable, and a meristem with such structure may continue growth unchanged almost indefinitely. This is notably the case when the "skin" formed from one component is only one cell layer thick, *i.e.*, it consists of dermatogen only. (Such periclinal chimaeras are said to be monochlamydius or haplochlamydeous.) Hardly less stable are periclinal chimaeras in which the "skin" is two cell layers thick (dichlamydius or diplochlamydeous). A meristem with a three-layered "skin" (trichlamydius) may also show a fair degree of stability, but the stability of meristems having "skins" of yet greater thickness is problematical.

It will be noted that in each of the above types two reciprocal forms are possible: one in which component A forms a skin over a core of component B, and the other in which B forms a skin over a core of A. The reciprocal forms of any type are often very different in appearance (*cf.* Winkler's *Solanum Tubingense* and *S. Koelreuterianum*), but apart from this they may differ in frequency of occurrence and in stability, *e.g.*, white-over-green variegation is commoner than green-over-white. Krenke (25) found that chimaeras with a skin of *S. memphiticum* over a core of tomato could be obtained with far greater ease than the reciprocal forms.

It should be clearly understood that the mature tissues derived from a periclinal meristem, while they show also a periclinal structure, do not necessarily possess a skin of the same thickness as that found at the growing point. The skin of the mature organs of a periclinal chimaera is always as thick as that of the meristem, but, on the other hand, in some species or varieties of plant it may be considerably thicker in certain regions.

It follows from this that the number of cell layers comprising the skin of the *meristem* (on which the designations monochlamydius, dichlamydius, etc., should be based), cannot be deduced with any certainty from examination of some *mature* organ. Failure to recognise this fact has often led to incorrect interpretation.

(D) Probably the chimaeral type that arises with greatest frequency in nature is that known as *mericlinal*. This has often the

superficial appearance of a sectorial chimaera, but differs in that the one component, instead of occupying a sector of the growing point, forms only a superficial strip or skin. It resembles, in fact, a periclinal chimaera in which the skin covers only a small part of the meristem instead of enveloping it as a whole. Only those buds arising at the edges of this strip will reproduce the mericlinal structure; those arising elsewhere on the strip will be periclinal; and those arising off the strip altogether will not possess a chimaeral structure but will consist of genetically uniform core tissue (see Fig. 1).

The majority of cases arising spontaneously in nature that have been reported as sectorial chimaeras were probably in reality mericlinal in structure.

It will be noted that whereas no change of pattern results on the production of lateral shoots in periclinal chimaeras, there is frequently in sectorial and mericlinal chimaeras a rearrangement of the components to form a periclinal pattern. It thus follows that after any considerable vegetative growth has taken place, all the chimaeral branches will tend to be of the periclinal type whatever was the pattern in which the components were arranged originally.

Examples illustrating these different types of chimaeras must be sought in the literature cited; space does not permit the inclusion of such descriptions here.

ORIGIN AND RECOGNITION OF CHIMAERAS

Recognition of a plant as a chimaera and determination of the type to which it belongs obviously turn on the possibility of distinguishing between the genetically unlike components of which it is built up. This may be often a matter of considerable difficulty since a differentiating genetical factor, even when present, may not become evident unless conditions are such that it can manifest itself.

For example, the limit between the two components of a chimaera differing in capacity or incapacity to produce chlorophyll is easy enough to determine in the foliage leaves, whereas in the meristem two such components are indistinguishable by direct observation. Again, if the components differ in respect to possession of a gene controlling the production of epidermal hairs, the presence or absence of such a gene is evident enough in the epidermal layer, but direct observation will not reveal its presence in the sub-epidermal

layer. Moreover, direct observation of the different layers of vegetative tissues will fail to detect the presence or absence of a gene concerned with floral characters. Such considerations make it likely that many plants may have a chimaeral structure without the fact coming to light except by chance or through investigation specially directed to reveal the fact. It is only those chimaeras in which the differentiating genes happen to be situated in tissues in which their effect can manifest itself that are likely to be readily recognised as such.

Apart from chimaeras artificially produced by grafting operations, those occurring naturally result mainly from somatic mutation. It has become evident that such mutations are by no means rare or exceptional occurrences. The genes show varying degrees of stability, some undergoing change, if at all, only under the influence of some powerful external stimulus; others having so little instability that they appear to undergo change spontaneously, the change occurring at any stage in the life history. Whenever a somatic mutation occurs, a potential chimaera is formed recognisable as such if the aberrant cell or cells multiply to form a tissue of a size and distinctness to attract attention.

Of recent years the anomalous behavior of certain plants has been accounted for on the hypothesis that they possess 'mutable genes,' i.e., genes that readily undergo change at any stage in the life history† (11, 18, 19). Some types of leaf variegation, for example, have been explained as due to such mutable genes or to mutable plastids which control chlorophyll development (20). The mutations occur locally during somatic development giving rise to albinotic areas of greater or less extent according to whether they occur early or late in differentiation. The resultant plants are chimaeras, according to the definition adopted here, in that they lack genetical uniformity; the instability and absence of a definite pattern in the disposition of the components, however, mark them off sharply from the foregoing classes of chimaeras.

In groups such as the ferns, in which growth is by a single apical growing point cell instead of by a cell complex, the periclinal type of chimaera is obviously not possible, but the occurrence of chimaeras arising by somatic mutation during development presents no theoretical difficulties. In point of fact, examples of such chi-

† See article on Unstable Genes by M. Demerec. The Botanical Review, Vol. 1, p. 233.

maeras have been described in both sporophytic and gametophytic generations of ferns (1).

The kind of differentiating characters of most particular value in tracing fully the distribution of the components of a chimaera are those which do not depend for their appearance on the morphological nature of the cells bearing them. Of such, those characters affecting the nucleus have proved far the most useful. The number of chromosomes in the nucleus is an obvious character of this kind and has been employed effectively on a number of occasions, e.g., in the analysis of the constitution of the *Solanum* chimaeras obtained by Winkler and subsequent workers. Even more valuable is difference in the size of the nuclei in the two components, since recognition of such a difference may be observed in resting nuclei. A case of this kind has been the subject of recent investigations by Krenke, the components being, respectively, *S. memphiticum* and tomato (25). In these chimaeras the distribution of the two components can be traced both in the meristems and in the different mature tissues with comparative certainty. This work is of considerable importance in that it has afforded support by analogy for interpretations offered in other cases based on indirect evidence and deduction rather than on direct observation.

Another cell character that has proved useful in this connection is the occurrence of chloroplasts. The advantage in this case is that the presence or absence of chlorophyll can be readily observed; much work has been expended, in consequence, in determining the distribution of chlorophyll-containing and chlorophyll-free tissue in variegated plants. In the majority of cases, though not in all, such distribution, as well as the behavior of the plants in other ways, can be accounted for on the supposition that the meristems possess a chimaeral structure so far as potentially green and colourless components are concerned. But it must be remembered that there is no *direct* way of distinguishing between the two components of a variegated plant in the meristem region. The structure of the meristem can be *deduced* from the arrangement of the components in the leaves or other mature tissues by analogy with chimaeras, such as those of *Solanum*, in which the relation between chimaeral patterns in meristem and mature tissues is known; supporting evidence may be derived from the nature of the sexually produced offspring or of the root cuttings; nevertheless, any opinion regarding

the structure of the meristem remains based on conjecture, however well this may be supported. Investigations on variegated plants, therefore, suggestive and interesting as they are, do not provide complete information, *based on direct observation*, as to the chimaeral structure of the plant as a whole, such as is provided by chimaeras possessing a nuclear difference in the two components.

Furthermore, it must be remembered that the production of chlorophyll requires the cooperation of two factors: (a) an appropriate gene inherited through the nucleus; (b) the presence of appropriate plastids distributed amongst dividing cells independently from division of the nucleus and in a very different manner. Caution is thus necessary in drawing analogies between the behaviour of variegated plants in which the components differ in a plastid-borne character, and chimaeras in which the components differ in respect to a character borne by the nucleus—although it may well be that the general rules governing chimaeral behaviour apply to both.

Variegation or irregular chlorophyll distribution in the leaves may be due to causes quite other than a chimaeral arrangement of potentially green and colourless meristematic layers at the growing point. Failure of chlorophyll to develop locally in tissues normally green may be physiological and due to nutritional factors, or may be a symptom of virus disease rather than an indication of genetical difference. Some types of leaf variegation behave genetically as simple recessives to green, and a number of cases have been explained on the hypothesis of mutable genes or mutable plastids controlling chlorophyll development, the mutations occurring locally during somatic growth and differentiation.

When the components of a chimaera differ in respect to characters conditioned in their expression by morphological position even more than is chlorophyll production, for example, the possession of epidermal hairs such as distinguishes peach from nectarine, still less dependence can be placed on direct observation as a means for elucidating the structure of the chimaera as a whole, and more reliance must be placed on deduction from analogy. Nevertheless, since study of the more thoroughly known chimaeras has shown that the range in their behaviour is between well-defined limits, there seems justification for assuming a similar behaviour in other cases so long as the known facts are in accord with such assumption.

Apart from direct observation of the tissues, information as to the chimaeral nature of the plant may be obtained in a number of ways:

(1) Root-cuttings, owing to the endogenous origin of lateral roots, offer a means of separating the core component in a pure condition.

(2) Sexually produced offspring, since the archesporium in flowering plants is formed from the hypodermal layer, provide plants pure for this layer, and consequently give information as to its nature. (Exceptionally, the archesporium is derived from the outermost layer.)

(3) Anomalous branches arising in the neighborhood of a superficial injury usually consist of core tissue which has "broken through" the skin. On the other hand, branches sometimes arise apart from any injury composed of the skin component alone, due to a local thickening of the skin in the region from which the bud arises. It must be remembered, however, that anomalous branches may sometimes arise as the result of a local vegetative mutation.

CHIMAERAS IN THEIR RELATION TO MORPHOLOGY

A study of chimaeras has proved useful in attempting to unravel problems of developmental morphology, because such studies have given precise information as to the particular layer of the meristem responsible for the different tissues of the mature plant.

In particular, much work has been expended in attempts to trace the exact ontogeny of the leaf. Such work is extremely laborious and difficult and the conclusions of different authors sometimes appear to be inconsistent. The following is an outline of the results that have been obtained,* with an indication of how inclusion of chimaeral types in the material studied supports the views arrived at.

Histogenetic studies of leaf formation suggest that the factors which must be taken into account in attempting to relate the different mature tissues of a leaf to definite regions of the growing point are somewhat as follows.

According to the careful investigations of Schmidt (38), the shoot apex consists of an inner core, the *corpus*, in which growth in volume predominates, covered by one or more layers of cells,

* For a more detailed treatment see article by Foster, Vol. II, No. 7, of this journal.

collectively the *tunica*, in which each layer is self-perpetuating since divisions of the constituent cells are anticlinal. Only during the formation of leaves or axillary buds do certain of these layers undergo periclinal division.

It may be noted here that the existence of such self-perpetuating layers is confirmed by the occurrence of stable periclinal chimaeras with one-, two- or even three-layered skins (25, 41).

The leaf primordia originate as folds on the stem meristem, the formation of these resulting from the occurrence of periclinal cell divisions in certain layers. No general statement can be made as to how many layers are involved. The investigations of some workers point to the second layer of the tunica alone being concerned (e.g., Herrig (15) in *Elodea*, *Galium* and *Hippuris*). Rösler (36) considered that in grasses, only the outermost layer, the dermatogen, contributes to the leaf tissues. Schmidt (38) found that in *Scrophularia nodosa* L. the tunica is a single layer (dermatogen), the leaf arising as a result of periclinal divisions in the outer portion of the corpus, while in *Vinca minor* L. there is a three-layered tunica, periclinal divisions in the two inner layers of this being responsible for leaf formation. The recent studies of Foster (13) suggest that in *Carya* the tunica is two-layered, the leaf arising from periclinal divisions in the inner layer of this and from the corpus. Further, Lange's investigations (12, 28) point to variation in behaviour between varieties of the same species, or even between young and mature stages of the same plant.

It is evident, then, as concluded by Foster (14), that no generalised statement can be made, but that "the mode of leaf formation is directly related to the architecture of the growing point in each particular case."

Even when the origin of the leaf primordium is known, the problem of tracing which part of the mature leaf is related to a particular layer of the stem meristem is solved only in part. The primordium is a protuberance having the form of a tapering cone flattened on the adaxial side and represents the future petiolar-midrib region. Two phases of development now occur:

(1) An increase in radial thickness as a result of meristematic activity of a vertical strip on the flattened adaxial side of the primordium. This activity occurs principally, it seems, in the sub-epidermal layer, the newly formed tissue constituting a sort of wedge on the adaxial side.

The structure of petioles of dichlamydius periclinal chimaeras lends strong support to this interpretation of what occurs. If, for example, the two photographs in Chittenden's 1925 paper showing transverse sections of the petioles of typical dichlamydius green-over-white and white-over-green periclinal chimaeras of *Pelargonium* be examined, the wedge of tissue derived from the meristematic activity of the second layer of the growing point is very evident in the upper side of the petiole, owing to its contrasting colour with the rest of the cortex derived from more deeply seated layers of the growing point.

(2) The leaf lamina begins to differentiate from the upper portion of the primordium by the organisation of two marginal ridges of meristem. As with the development of the leaf primordium from the stem, so here there is great variation in regard to the number of layers taking part in meristematic activity. Thus, Krumbholtz (26) and Lange (27) contend that both subepidermal and deeper layers may participate in varying degrees; others, such as Avery (3), Johnson (21) and Weidt (39), among more recent workers, consider the subepidermal layer mainly or exclusively responsible. Renner (33) finds that the edge of the leaf of *Sambucus nigra* results from periclinal divisions of the outermost layer (dermatogen), as was found to be the case in certain monocotyledons by Pottier (32). Foster (13a) has also found this in bud scales of rhododendrons.

The anomalous behaviour of certain chimaeras, such as *Pelargonium* "Freak of Nature" and *Hydrangea hortensis nivalis*, was accounted for by Chittenden in 1925 (7) by the hypothesis that the dermatogen underwent periclinal divisions during the formation of the leaf margin in these varieties. Such chimaeras thus lend support to Renner's finding in the case of *Sambucus*.

From this brief summary it will be evident that the problem of tracing back any particular tissue of the mature leaf to its point of origin in the stem meristem is one of extreme difficulty, since the development of the tissue in question must be followed through two phases of meristematic activity, firstly, that leading to the formation of the leaf primordium, secondly, that leading to the development of the lamina from the primordium.

If, however, the plant under investigation can be obtained in the form of periclinal chimaeras of known structure, the task becomes simplified and the results more certain. Thus, examination of a

monochlamydius form would show that the outer layer of the tunica (dermatogen) gives rise normally only to the epidermis of the mature plant (*e.g.*, in the studies of *Solanum* chimaeras by Lange (27) and Krenke (25)), although exceptionally more deeply situated tissues in certain regions may be derived from this source (*e.g.*, the mesophyll of the leaf margin in *Sambucus nigra* (33), *Hydrangea hortensis nivalis* (7), etc.). Similar examination of a dichlamydius form would show that the second layer of the tunica is normally responsible for the production of the subepidermal layer covering the plant, and in certain regions may be responsible for much more—for almost the whole of the mesophyll of the leaf except that in the centre or round the vascular bundles.

The final contribution of each of the layers of the tunica towards building up the mature leaf appears to be shown with dramatic simplicity in those chimaeras in which the two components differ in respect to presence or absence of chlorophyll. But while most of the deductions that have been drawn from the distribution of chlorophyll in variegated plants are no doubt justified, as pointed out previously, it should not be lost sight of that this character is of rather a special nature, and that the actual structure of the growing point is based on inference only.

It may be noted here that the structural principles on which periclinal chimaeras are built up, as set out earlier in this article, do not agree altogether with the views expressed by Y. Imai based on examination of a number of variegated types (20).

According to this worker, "the majority of dicotyledons are usually composed of three histogens, and *Pelargoniums* have this constitution. . . . Monocotyledons consist of two histogens." These histogens he designates ecto-, meso- and endohistogen, only the first and last being present in monocotyledons. Apparently, he does not regard any of these except the ectohistogen as a *single*, self-propagating layer of cells, nor contemplate the possible existence of more than three self-propagating units. He simplifies the interpretation of those variegated pelargoniums which owe their variegation to a chimaeral arrangement at the growing point by the following generalisations. (1) the ectohistogen contributes to the epidermis and marginal mesophyll of the leaf; examples given being "Freak of Nature" and Chittenden's "Variety A" (7) which represent forms in which the ectohistogen is respectively green and colourless; (2) the mesohistogen contributes the outer mesophyll

and submarginal parts of the leaf; *e.g.*, the commonly occurring green-edged and white-edged forms in which the ectohistogen and mesohistogen are both either green or colourless, respectively. Examples of the two remaining possible types in which the mesohistogen is green or colourless while the ectohistogen and endohistogen are at the same time colourless or green are not mentioned.

While something may be said for this attempt towards simplification, the very diverse conditions recorded by other workers to occur in different species makes it doubtful whether any generalised statement is possible. The descriptions and photographs of the growing points of his *Solanum* chimaeras given by Krenke (25) leave little doubt that in these at least each of the three outer layers has a definite individuality, the condition approximating more closely to the series of self-perpetuating layers, each one cell in thickness, described by Schmidt (38) under the term 'tunica,' overlying a central 'corpus,' than to the ecto- meso- and endohistogens of Imai's generalisation. As to *Pelargonium* in particular, as Chittenden (8) remarks, the development of the leaf has been observed by several workers, and whatever their differences, all agree that the marginal mesophyll is derived from the subepidermal layer of the growing point and not from the dermatogen. Moreover, Baur (4) states that among his chimaeral forms of *Pelargonium* were some entirely green but with a "colourless" epidermis (judged from the guard cells), and others entirely colourless except for a "green" epidermis, without the leaves bearing a conspicuous colourless or green margin as would be expected if the dermatogen contributed to the formation of the mesophyll. Until further evidence is available, therefore, it would appear preferable to accept provisionally the view that the dermatogen normally gives rise only to the epidermis of the leaf in *Pelargonium* and *Pelargonium* chimaeras (as in the chimaeras of *Solanum*, *Cytisus*, *Crataegus*, etc.) ; and that the production of the marginal mesophyll also from the dermatogen is an individual peculiarity of certain varieties like "Freak of Nature," as suggested by Chittenden, rather than a general feature of the genus as postulated by Imai.

ECONOMIC IMPORTANCE OF CHIMAERAS

Hitherto the only class of chimaeras that has achieved economic importance includes those periclinal forms built up of green and colourless components which are prized for their ornamental varie-

gated foliage; a few others, such as *Cytisus Adami* and the *Crataego-Mespili*, are sometimes carried in nurserymen's stock and find their way into private gardens as interesting curiosities rather than for their decorative value.

The periclinal form of chimaera undoubtedly possesses, however, potential value in relation to disease resistance. The chances of infection of a susceptible variety of plant would be greatly lessened, one would suppose, if the variety in question could be obtained as a periclinal chimaera in which the skin was contributed by some immune variety. If the plant were one readily propagated vegetatively, the immune chimaeral form might well become commercially important.

Jørgensen (22) in 1927 recorded an attempt to produce a potato chimaera with a skin of tomato immune, as is tomato, to attack by *Phytophthora*. In the course of his experiments two chimaeras were obtained, but unfortunately both consisted of tomato with a skin of potato. Moreover, although the experiments show that periclinal chimaeras between potato and tomato are possible, they suggest also that the combination may not be a very happy one since the plants failed to flower or survive beyond one season. It is possible, nevertheless, that the reciprocal forms, should they be obtained, might prove more amenable to vegetative propagation, as they would presumably yield tubers. Krenke (25) reported that he was engaged in the attempt to obtain a chimaera between two varieties of potato of which that forming the skin was to be an immune variety—a possibility envisaged by Winkler. Up till now success has not been recorded.

Investigations by T. Asseyeva in 1928 (2) and by M. B. Crane in 1936 (10) suggest that nature may have already achieved the objective aimed at by Krenke. By the simple process of removing the eyes from potato tubers to a depth of about 1 mm. and thereby inducing adventitious buds to form from internal tissues, Asseyeva was able to show that a number of varieties commonly grown in Russia have a chimaeral constitution. A similar technique was employed by Crane on the variety "Golden Wonder" which has a thick brown russet skin. His results prove that "Golden Wonder" is a periclinal chimaera with a core of "Langworthy," a variety with a smooth skin. Since the russet skin character does not appear in seedlings of "Golden Wonder," the plant is evidently a monochlamydius periclinal. McIntosh (29) states that the same geneti-

cal behaviour is found in "Field Marshall," another russet-skinned variety, which, therefore, presumably has a similar chimaeral constitution.

These observations suggest that many of the varieties of potato that have arisen as somatic variations are probably periclinal chimaeras, and it is obviously desirable to investigate susceptible and immune varieties with a view to ascertaining whether the immunity of the latter can be correlated with the possession of a chimaeral structure in which the skin component provides a resistant barrier for more susceptible core tissue beneath.

The immunity reactions of a periclinal chimaera may not be of the simple nature suggested in the previous paragraphs. There are two alternatives to be reckoned with:

(1) Each component of the chimaera may retain its own characteristics in respect to infection by pathogenic organisms unmodified by the presence of the other.

In such a case, a periclinal combination with susceptible core and immune skin should be resistant (this assumption is the basis of Jørgensen's and Krenke's experiments), while the reciprocal periclinal form should be liable to infection.

Support for the existence of this simple relationship is given by experiments which show that in grafted plants scion and stock retain their individual characteristics. Thus, in sunflower-artichoke grafts, as recorded by Colin (9) and others, inulin is found only in the artichoke tissues whether artichoke is used for scion or stock. Similarly, there are many instances recorded in which immune and susceptible plants have been grafted and both scion and stock have retained their characteristic immunity reactions. Examples of such are *Mespilus germanica* (immune) on *Crataegus oxyacantha* (susceptible) when infected by *Gymnosporangium confusum*, and *Sorbus aria* (immune) on *S. aucuparia* (susceptible) when infected by *G. tremelloides*, quoted by Fischer (12) and Sahli (37); and there are many subsequent experiments with other plants which have given corresponding results.*

(2) Alternatively, the components forming the chimaera may each modify the other's immunity reactions. This modification could operate in several ways:

* For a detailed discussion of "Disease relationships in grafted plants and chimaeras" with citation of literature, see T. E. T. Bond, Biological Reviews, 11, 1936, p. 269.

(a) The skin of a susceptible variety over a core of an immune variety might have its resistance raised by contributions of immunising substances from the large bulk of immune tissue with which it is in contact. Reference may be made to p. 256 of the article by W. Brown on the "Physiology of Host-Parasite Relations," Vol. 2, Botanical Review, for a discussion of the chemical factors that may be concerned in such cases.

(b) In the reciprocal combination, the immune skin might have its resistance lowered by contact with the susceptible core.

(c) Apart from transmission of substances responsible for conferring immunity directly to the tissues, lack of balance in the nutritional requirements of skin and core might lower the general vigor of the combination, or of one or both components, and so render the chimaera more susceptible to pathogenic attack than the nature of the skin component would lead one to expect.

So far as (a) and (b) are concerned, it has already been pointed out that there is a large body of evidence showing that characteristic metabolic products are not normally interchanged between scion and stock nor are the immunity reactions modified; the same relations will presumably hold between the components of a chimaera. At the same time there are exceptions to this generalisation, so that the possibility of such action cannot be left entirely out of account. For example, atropine and nicotine have been observed to pass from *Atropa belladonna* to *Solanum tuberosum* and from *Nicotiana tabacum* to *N. affinis*, respectively (34). The complete facility with which growth-controlling substances or auxins from one species of plant will pass through the tissues of another likewise shows that the transmission of organic substances from one plant through the tissues of another is sometimes possible. Direct transmission of a capacity for resistance or of susceptibility between scion and stock receives little support from experiment, however, although it has been claimed by certain workers in the case of crown gall infection with some appearance of justification. (16, 35, 42).

In regard to (c), it can hardly be doubted that in some chimaeras, as sometimes between scions and stocks, there are varying degrees of incompatibility.

A good deal of attention has been devoted in recent years to the properties of different stocks in so far as they affect fruit trees grafted on them as scions, and it is now realised how profoundly the natural characteristics of a variety may be modified by way of

control of water supply, proportion of mineral salts, etc., on the part of the stock. By such means the nature of the stock may alter the physiological reactions of the scion towards the environment and be responsible for various physiological disorders affecting quality and storage-life of the fruit. Thus, liability to leaf scorch (17) and early breakdown during storage of fruit in Bramley's Seedling (23) on certain stocks are probably correlated with deficiency of potassium. In this indirect manner the resistance reactions of a scion might easily be modified without the necessity of predicated the transmission of any specific substance conferring immunity. Similar relations might be expected to hold between the components of a chimaera.

Inoculation experiments with different periclinal chimaeras have provided definite evidence of mutual reaction of the components on one another, probably indirect, although the possibility of direct action is not excluded.

Thus, in the experiments of Klebahn in 1918 (24) Winkler's chimaeras were infected by *Septoria lycopersici*, a parasitic fungus which attacks tomato but not nightshade. *Solanum Gaertnerianum*, having a growing point with two layers of nightshade over a core of tomato, proved immune, whilst *S. proteus*, the reciprocal form, was susceptible—results which might be expected when it is borne in mind that a large part of the mesophyll of the leaf is derived from the second layer of the meristem and so consists of nightshade in one case and tomato in the other. On the other hand, *S. Koelreuterianum*, having a growing point with one layer of nightshade over a core of tomato and producing leaves wholly of tomato tissue except for the epidermis, was susceptible; the single layer of nightshade tissue appeared inadequate to provide protection; in other words, the immunity reactions of the plant were not determined by the nature of the epidermis alone.

Similar infection experiments by Fischer in 1912 (12) and by Sahli in 1916 (37) on *Crataego-mespilus* chimaeras with *Gymnosporangium confusum* showed that these plants were susceptible, although the core of susceptible *Crataegus oxyacantha* is covered by a skin of immune *Mespilus germanica*; the degree of susceptibility of the chimaeras was less, however, than that of the *Crataegus* component.

In later experiments, Maurizio (30) claims that when *Podosphaera oxyacanthae* was used as the infecting organism,

Crataego-mespilus Dardari (probably a dichlamydius form in which the epidermis and much of the leaf mesophyll consists of *Mespilus*) proved slightly susceptible, and *C. Asnieresii* (having the epidermis only of *Mespilus*) proved entirely immune, although both *Mespilus* and *Crataegus* are readily infected. Other experiments in which *Piro-cydonia Winkleri* was inoculated by *P. oxyacanthae* showed this plant to be more susceptible than either pear or quince, of which two species it is considered to be compounded. Such results as these point to mutual reactions of the chimaeral components that may affect their natural immunity and to the need for caution in attempting to forecast precisely the degree of resistance of a chimaera to pathogenic attack.

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THE DEVONIAN FLORAS AND THEIR BEARING UPON THE ORIGIN OF VASCULAR PLANTS

OVE ARBO HÖEG

Trondheim, Norway

INTRODUCTION

Two decennia have elapsed since publication of the first part of the series of monographs in which Kidston and Lang described the silicified plants discovered in 1913 in the chert bed at Rhynie in Scotland. The minute knowledge of the anatomy and morphology of these marvellously well preserved plants threw a brilliant light on many other forms, previously described but imperfectly understood, and stimulated further research. From that time Devonian floras¹ have attracted more interest among paleobotanists than has any other subject, and, although no occurrence has been discovered that can compete with the Rhynie plants as to preservation, these years have meant an immense increase in our knowledge of the oldest terrestrial vegetation. This is due chiefly to research in the British Isles, Germany and Australia.

The scope of the present article, written not for the specialist but for the general botanical reader, is to give a review of the most important Devonian floras and of the problems they solve or raise as to the origin of vascular plants.

REGIONAL AND VERTICAL DISTRIBUTION OF THE OLDEST VASCULAR PLANTS

It was Arber who first clearly pointed out, and overestimated, the difference between the Upper Devonian *Archaeopteris* flora and the preceding *Psilophyton* flora. At his time the many remarkable Middle Devonian plants were scantily known. To some extent they form a transition between the vegetation of the Lower and the Upper Devonian and serve to lessen the contrast, which to a certain degree may be due to ecological conditions. But upon the whole it remains unblurred as one of the most radical changes in the history of plants.

¹ For a geological time scale the reader is referred to page 417 in volume 2, 1936, of the Botanical Review. Citation No. 43 of this article.

While the Upper Devonian flora, in spite of certain characteristic forms of restricted vertical range (*Archaeopteris*, *Pseudobornia*, *Cyclostigma*), in its essential features is compatible with that of the Carboniferous, although poor in comparison, it is in the lower and middle parts of the formation that we find the many interesting plant forms which one may hope will throw light upon the origin of the groups dominating in later times.

Plants of the Lower and Middle Devonian, rarely of the Silurian, are now known from nearly all parts of the globe. Their world-wide distribution in the Lower Devonian, and the abundance of different forms of relatively high organisation, make it probable that the floras known to us are the result of a long preceding evolution. This assumption is not necessarily correct, however, a sudden rise of a plant group being known in various geological periods — compare the short time which the living families of angiospermous trees seem to have needed for a complete conquest of the world (although we do not know what went on previously in regions from which no fossils are preserved). As to Devonian floras, the evolution of terrestrial plants (and animals) was long regarded as possibly having been caused by the wide regression of the oceans in Downtonian and early Devonian times, particularly in the area of the Old Red Sandstone. It is still quite probable that the creation of new species was favoured by physical conditions in the large and partly inundated plains where this sandstone was formed, but the fact that terrestrial plants have been found outside these areas has shown that the adaptation of plants to life on dry land did not start then and there. Furthermore, the discoveries in Australia related below have proved the existence of vascular plants of surprisingly high organisation even early in the Silurian. The origin of vascular, terrestrial plants cannot at present be explained through any geological causes.

PSILOPHYTALES, THE CENTRAL GROUP OF DEVONIAN PLANTS

To many botanists and geologists the name of Devonian plants has become very nearly synonymous with that of psilophytes. This association is decidedly too strong because even the early Devonian floras comprise plant forms which can not be included in the said group, forms which represent entirely independent lines of development, such as *Prototaxites*, *Pachytheca* and *Parka*, and others

42. b may be more or less related to the psilophytes without be-

longing to them, such as *Hyenia*, *Baragwanatia*, (?) *Schizopodium*, and still more distant forms. Whether all the vascular plants of that time represent a monophyletic transgression from water to dry land is a question which can not be definitely solved at present, but which will be touched upon in the following pages.

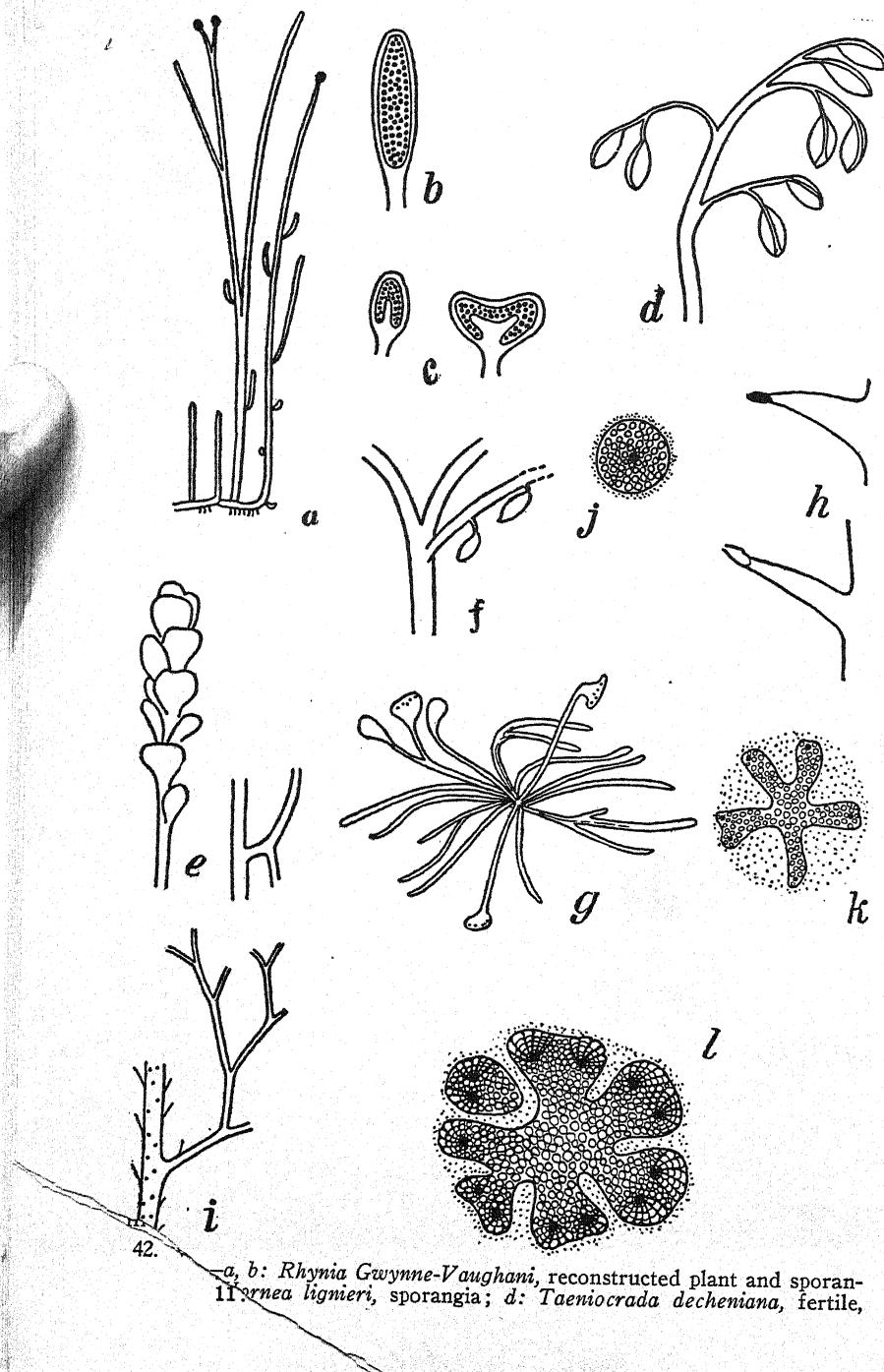
The psilophytes, of which no typical representative seems to have outlasted the transition from Middle to Upper Devonian (or possibly a slightly higher stratigraphical point), are characterised by the position of the sporangia, terminal on stems and branches (telomes), and by the lack of real roots and leaves. But in other features, as anatomy, organisation and habit, there is great diversity which becomes more evident every year.

Rhynia may be regarded as a central type (whether it is really primitive, or reduced, is of minor importance in this connection, but will be discussed later), and as a parallel to it, no doubt closely related, stands *Hornea*, differing especially in having columellate sporangia. These genera (Fig. 1a-c, j) have not been found with certainty outside the Rhynie Chert Bed in the Middle Devonian of Scotland. As is well known, they had a horizontal rhizome (articulate in *Hornea*), with erect, cylindrical, spineless stems, branching sparingly in simple dichotomy, and bearing isosporous sporangia which had no elaborate arrangement for dehiscence, terminal on the main stems and branches; they possessed stomata of an archaic form, and a thin cylindrical protostele of the simplest construction possible, the central protoxylem usually slightly different from the surrounding metaxylem. From this simple type there are, generally speaking, two lines of development.

I

One of these lines leads to further specialisation of the spineless type. The most important forms are:

Taeniocrada decheniana (Fig. 1d) (28). Was a band-like and evidently flexible plant with a maximum breadth of 1.5 cm., dichotomously branched, tracheides. This external form and the lack of stomata could scarcely be understood without the assumption that the plant lived submersed in water (according to Kräusel and Weyland). The sporangia were borne in clusters which are also supposed, with some reserve, to have been subaquatic. The species (formerly known as *Haliserites*) is widely distributed in the Lower Devonian of the Rhine, and has been reconstructed by Kräusel and Weyland. The genus seems to have been wide-spread in Europe and North America.



Zosterophyllum (Fig. 1e) (8, 33, 34, 37) was smaller, about 15 cm. high and a few millimeters broad, and had a gregarious and tufted growth. It branched freely with dichotomous and lateral branches. Particularly in the lower parts it had a very characteristic mode of ramification, the lateral branches just after leaving the main stem very often giving off a secondary branch in a downward direction. Central strand very thin, composed of tracheides with annular thickenings. Cuticle thick. Presence of stomata possible, but not well known. Many of the branches were clustered at the base of the plant, as if forming a rhizomatic (or stoloniferous) part of it; others were erect, and generally in their upper portions they bore lateral, stalked, reniform sporangia, arranged spirally in a kind of long spike. The sporangia opened tangentially along the upper margin and contained only one kind of spore, 25-30 μ in diameter. This description (after Lang) applies chiefly to *Z. myretonianum*, the earliest vascular plant of Great Britain, from the Lower Devonian and probably Upper Downtonian. Other species have been reported from the (probably Lower) Devonian of Australia, the Lower Devonian of the Rhine Area, and (?) from the Upper Devonian of Maine. A type of fructification resembling *Zosterophyllum* is *Bucheria*, described from the Lower Devonian of Wyoming by Dorf (9, 10).

Gosslingia breconensis (Fig. 1f) (16), from the Lower Old Red of Wales, differs from the preceding genus in having circinate tips and a relatively strong vascular bundle (probably with exarch protoxylem). They are regarded, however, as having been closely related. The sporangiferous branch seemed to leave the axis from immediately below a point of bifurcation; sometimes in the same place there is only a projection instead of a branch (compare the "axillary buds" of *Hostimella*).

Hicklingia (23, 27) from the Middle Old Red of Scotland and (?) the Middle Devonian of the Rhine area had the same tufted growth and naked axes, but differed above all in the sporangia being large and terminating the upper branches.

These Psilophytales are older than *Rhynia*, and at first glance more alga-like, particularly *Taeniocrada*. If the latter, however, was really a subaquatic plant, it must have represented a secondary adaptation, because a plant with tracheides and sporangia of this type must have been descended from terrestrial ancestors. At all events, the fertile regions of the genera in question are on a much

looking very much like an alga, but with a thin central strand-containing shoot; e: *Zosterophyllum*, fertile shoot and dividing branch; f: *Gosslingia*, branch bearing two sporangia (details uncertain); g: *Sciadophyton*, entire plant; h: *Psilophyton princeps*, spines; i: *P. goldschmidii*; j, k, l: diagrammatic cross-sections of stele of j, *Rhynia*, k, *Asteroxylon*, l, *Schizopodium*; protoxylem black, metaxylem circles, phloëm dots. (Not drawn to scale. Based upon drawings and photographs by: a, b, c, j, k, Kidston & Lang; d, g, Kräusel & Weyland; e, Lang, Cookson, Kräusel & Weyland; f, Heard; h, Lang; i, Halle; l, Harris.)

higher level of organisation than in *Rhynia* with regard to both the branching of the sporangiferous shoots and to the structure of the sporangia themselves.

As the largest and most highly organised member of the spineless psilophytes may be mentioned *Pseudosporochnus* which, after Kräusel and Weyland's re-examination of the Bohemian Middle Devonian flora (31), belongs to better known Devonian plants.

Pseudosporochnus from the Middle Devonian of Bohemia, Scotland, and possibly Norway, is one of the largest known psilophytes, attaining a height of about one meter, perhaps more. Stem erect, stout, dividing by rapidly repeated dichotomy into a number of main branches which through further ramification form a great number of ultimate divisions, bearing, in some places, small sporangia containing minute spores.

Pseudosporochnus is of interest as showing the most extreme development in a certain line of psilophytes, but our present ignorance as to its anatomical structure makes any further phylogenetic inference unsafe. Although after the reconstruction by Kräusel and Weyland a comparison with macrophyllous fern-like plants might possibly be discussed, it seems to be more probable that the plant represents a phylogenetic cul de sac.

Before we leave this group two problematic plant forms may be mentioned:

Sciadophyton steinmanni (Fig. 1g) (28) from the Lower Devonian of Germany (a related species, less well known, occurs at Gaspé) was a small plant, rosette-like, with little stems growing out from one point on the ground; they were maximum 8 cm. long, usually simple but sometimes dichotomously branched, traversed by a fairly strong strand in which there seem to have been tracheides. Distally, many of the branches widened into a short cone or disc on which there were globular bodies, possibly sporangia.

This little plant was probably vascular, but beyond that its position, as stated by Kräusel and Weyland, will remain entirely uncertain until the nature of the apical organs has been cleared up. Attention may, however, be drawn to the similarity between the latter and the organs supposed to be sporangia in *Dutoitia pulchra* (17). In this South African plant the presence of vascular elements has not been demonstrated; on the other hand, it has short spines which give its dichotomous stems an aspect entirely like that of a psilophyte. It is quite probable that these two genera were closely related, and in view of the spines of the one and the tracheides of the other it is natural to regard them as psilophytes. It is probable that they will one day help to clear up some phylo-

present they form only an unsafe basis for genetic question.
speculation.

Sporogonites will be ed later.

II

within the Psilophytale is characterised by the presence of spines, though a question remains as to the hemispherical protuberances.

Comparison suggested beneath a stoma, and from which of *Rhynia*, which often reaches (in *R. Gwynne-Vaughani*) the adventitious deciduosity, these are of a different nature. Doubtless small, irregularly placed emergences, consisting of a less hemispherical cell-complex and terminating in a hair, apparently consisting entirely of cuticle. Even if these features are not such as might be expected in a phylogenetically early stage of, for example, the emergences of *Psilophyton*, they at least tend to lessen the contrast between the naked and the spiny psilophytes.

Unfortunately, the genus *Psilophyton* is not among the best known of its relatives, and this is true also of the type species, *P. princeps*, which Dawson described in 1859. His restoration of 1882 has been confirmed in its essential features by later investigators, and important details have been added lately, above all by Edwards in 1924 (11), and Lang in 1931 and 1932 (35, 36).

Psilophyton princeps is supposed to have had a creeping rhizome, although the connection between the fossil parts regarded as rhizomes and the rest of the plant has not been definitely demonstrated. It had erect stems which were scarcely of the thickness of a pencil and probably may have attained a height of about half a meter. The thicker parts of the stem were sparingly branched in more or less regular dichotomy, while the thinner branches divided more frequently, with tips often recoiled. Concerning the inner structure, Dawson described a central cylindrical strand of scalariform tracheides. The stomata were surrounded by cuticularised ridges. Stems and main branches densely spiny. The spines (Fig. 1*b*), which have been studied in marvellous preparations by Professor W. H. Lang, were 3-2 mm. long, without stomata, and ended in a swollen tip, showing that these emergences were probably of a glandular nature. In most cases the head was dark as if still filled with some kind of secretion, while in others it was empty. Together with the spiny branches occur others which are much more smooth, sometimes quite naked, in other cases bearing a few spines, and often longitudinally grooved. It is probable that

they represent the ultimate ramifications of the same sporangia often bear mm. long and 1–1.5 mm. wide, probably without any arrangement for dehiscence, the wall consisting of a double layer of ^{ad.} spores are numerous, and of uniform size, in flattened condition.

P. goldschmidtii (12, 28) differs particularly in lateral branch systems which are flattened and spineless, the spreading out in one plane (Fig. 1*i*). The species was described by (Röragen) and seems to be wide-spread, at least in Europe.

Asteroxylon of the Middle Devonian is a genus of two species, one of which, *A. mackiei*, from the Rhynie Chert, known in minute detail as to internal structure, whereas the exteⁿt has to be reconstructed chiefly from thin sections of the fossil chert. The other species, *A. elberfeldense*, from the Rhine Diⁿ and several localities in Scotland, is known primarily as impressions, above all, the external appearance, but also some anatomical details. The plants were relatively large, attaining a height of one meter or more, rising from (subaquatic?) rhizomes, measured about 1 cm. in diameter and were there clad with numerous adpressed "leaves" (*Thursophyton*). Higher up they became thinner and the leaves fewer and less adpressed and the thinnest parts were entirely naked (*Hostimella*). The stem had a central strand, containing in the German species a parenchymatous pith but not in the Scotch species. The stele (Fig. 1*k*) had an inner body of xylem, consisting of tracheides with spiral thickenings (or, in *A. elberfeldense*, of even a more complex structure). In cross-section the xylem was stellate, the protoxylem being situated within the widened ends of the lobes. The phloem formed a sheath between the lobes and around the stele, which, as a whole, was circular in outline. From the ends of the lobes the stele gave off slender bundles which passed through the parenchymatous cortex, each stopping at the base of a leaf without entering it. The epidermis had stomata of a rather specialised type. The "leaves", which attained a length of 1 cm., consisted of parenchyma, and in *A. mackiei* had a few stomata, but evidently none in *A. elberfeldense*. Sporangia were borne on slender, smooth branch-systems; in *A. elberfeldense* they are very imperfectly known, whereas sporangia in all probability belonging to the other species were pear-shaped, about 1 mm. long, with apical dehiscence by means of an annulus resembling that of the ferns (Zimmermann), and with only one kind of spore, developed in tetrads about 64 μ in diameter.

RELATION OF THE DEVONIAN PLANTS, AND PARTICULARLY OF THE PSILOPHYTES, TO THE MAIN GROUPS OF VASCULAR CRYPTOGAMS

The morphology and phylogeny of the sporophyte of vascular cryptogams have been fully treated by Lady Isabel Browne (6) in a recent paper in The Botanical Review. Only a few words need be said here on the question in general.

Speculations flourished on the morphology of plants and on the origin of various organs, chiefly the relation between leaf and stem, during parts of the 18th and 19th centuries, one of the most important early contributions to Nature Philosophy being that of Goethe. But in our day it is scarcely necessary to go further back than to the French paleobotanist Lignier and to his publications from the first decennium of our century.

In Lignier's view, the most primitive terrestrial ancestor of vascular plants had a small stem which divided in regular dichotomy; later, the two branches from a point of division might have become unequal, the one taking the main direction of the stem and pushing the other aside as a lateral branch. There was no root but a forked rhizome, morphologically not different from the stem. There were, at first, no leaves, but these came into existence through development in two different ways: (1) From "phylloids", small lateral appendages or emergences which appeared secondarily on the stem and which were transformed into small leaves. This would be the origin of the leaves of lycopods. (Such "phylloids" are comprised in one concept of "enations", as defined by Bower [5], together with hairs, scales, prickles, etc.) (2) From "cauloids", lateral branches with limited growth, the divisions of which began to arrange themselves in one plane. They became flattened, and by lateral fusion of a number of such cladodes broad leaf-blades might have been formed; such a fusion is now termed "webbing". This evolution, to continue the quoting of Lignier, characterised the origin of the vast majority of vascular plants. In some plants the leaves developed excessively at the cost of the stem; this was the macrophyllous stock, comprising all plants of the habit of ferns and cycadophytes. Along another line of development the leaf became reduced in size; these are the microphyllous plants: conifers, *Cordaites*, *Ginkgo*, and others. (It should be noted that the word microphyllous later acquired a somewhat different meaning, denoting all small-leaved groups, including lycopods and the Articulatae.) A third group would be the mesophyllous plants, in particular, the angiosperms, and finally, along a separate line which left the macrophyllous one at an early point, the Articulatae would be derived. In the latter, the characteristic features are, chiefly, reduction in size of leaves, their verticillate arrangement and their lateral union so as to form broad laminae or sheaths.

Lignier based his hypothesis partly on the morphology of *Psilophyton princeps*, in so far as the plant was then known through papers by Dawson. The knowledge of Devonian plants which increased tremendously after that time has confirmed his ideas in general. A salient point in them is the biphytic origin of the leaves, and already in 1916 Halle was able to show that *Psilophyton goldschmidii* may really be regarded as representing, in one plant, both of Lignier's leaf types in the initial stage. The same ideas have formed the point of departure, more or less directly, for most theoretical considerations of Devonian plants until to-day. Some paleobotanists, however, among them Zimmermann (45), hold a different view and regard microphylls and macrophylls as organs of the same origin, both developed, respectively, through reduction and augmentation of the size of axial units, telomes.

Let us now inquire what members or ancestors of the main groups of vascular cryptogams are to be found among Devonian plants, and particularly their relation to the psilophytes. The angiosperms will not be included here; reference may be made to another recent article in this journal (43).

LYCOPODIALES. The habit of the spiny psilophytes, particularly *Asteroxylon* in its *Thursophyton* stage, has a strong resemblance to a modern club-moss, and this resemblance is not merely superficial, but is equally striking in the anatomy of the stem. In this connection it is also of interest to note that in *Lycopodium* the anatomical difference between the stem and the root is comparatively small; in this character, *Lycopodium*, more than any other living vascular plant (except the Psilotales), has preserved a primitive feature. It is questionable, however, how far back the genus *Lycopodium* and its closest relatives are to be traced in geological history. Most of what has been named *Lycopodites* does not have much to do with the modern genus, and besides, the dominating members of the group in Paleozoic time were ligulate. The latter must, however, have descended from eligulate forms. Such forms are really known for there are at least some trustworthy species of *Lycopodites* in the Carboniferous, and in *Cyclostigma*, which is common in the Upper Devonian, no ligule has ever been observed, perhaps because it never was there.

With regard to vegetative characters only, there would scarcely be any difficulty in proclaiming the spiny psilophytes as ancestors of the lycopods, or at least as closely related to their ancestors. The difficulty arises from the fertile region. In all lycopods the sporangia are situated on the upper side of the leaves or at the leaf bases, whereas in all psilophytes which are known so far, they are terminal on axial organs. A psilophyte with sporangia in the axils of spines would help to bridge the gap; therefore, special interest is connected with the genus *Drepanophycus*.

Drepanophycus spinaeformis Göpp. (= *Arthrostigma gracile* Daws.) (12, 28, 33, 36) from the Lower Devonian of Canada, Scotland, Norway, Germany, China and Australia usually had a very characteristic appearance, with thick stems, in flattened condition up to 3.5 cm. broad, and sometimes giving off thick branches, which at first were horizontal and then suddenly turned upwards; in other cases they divided dichotomously. The impressions often bear traces of a thin central strand in which Halle, and later on Lang, could prove the existence of annular tracheides. Stomata are also known. The spines were very remarkable, being large but rather varying in size, broad and triangular at the base, resembling, more or less, rose thorns. They contained a nerve, connected with the central strand by a leaf-trace. The thinner axes may be difficult to distinguish from *Psilophyton*. This fact is all the more surprising because there is a profound difference in the reproductive organs, which have become known through recent investigations by Kräusel and Weyland in the Lower Devonian of western Germany. The sporangia were rounded bodies, each attached by means of a short stalk to the upper side of a spine not far from its base (Fig. 2b). Spores about 26-45 μ .

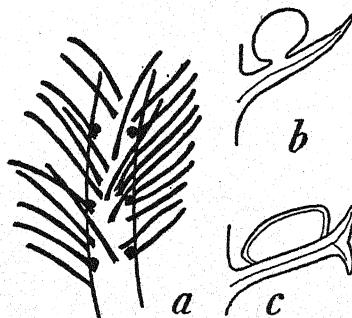


FIG. 2.—a: *Baragwanatia*, with sporangia; b, c: sporophylls of b, *Drepanophycus*, c, *Protolepidodendron*. (Not drawn to scale. Based upon drawings and photographs by: a, Lang & Cookson; b, c, Kräusel & Weyland.)

For a long time *Drepanophycus* was regarded as so closely related to *Psilophyton* that the distinction between them was difficult,

and really most of its vegetative characters may well be interpreted as indicating psilophytalean affinities. The position of the sporangia, however, certainly makes it impossible to retain it within that group, without widening its limitations more than is justifiable, and denotes, on the other hand, an approach toward the lycopods. Perhaps it may be so expressed that, besides peculiarities of its own, *Drepanophycus* presents characters in common with the psilophytes, and others in common with the lycopods. To this extent it serves to connect the two groups.

Drepanophycus does not stand alone. Together with a number of other forms recently described it constitutes a group that may perhaps be less heterogeneous than the diversity of its habit suggests. Only one will be described in some detail here:

Baragwanatia (Fig. 2a) was described in 1935 by Lang and Cookson (38) from material from the Silurian of Victoria, Australia. The only species, *B. longifolia*, had stems mostly 1-2 cm. broad, bearing numerous spirally arranged simple leaves which were firm but not stiff and rigid; they may have attained a length of 4 cm. and were uniform in width, about 1 mm., from the expanded base to the blunt tip. The reniform sporangia, about 2 by 2 mm., were situated among the leaf insertions in certain zones of the ordinary shoots, possibly adaxially, near the bases of leaves. Spores 50 μ . Large central cylinder with primary xylem, stellate in cross-section and composed of uniform tracheides with narrow annular thickening. The leaves contained a bundle connected with the central cylinder by leaf traces. Epidermis and stomata unknown.

This plant is remarkable not only for its great age but still more because of its organisation. The authors compare it with *Drepanophycus* and regard it, certainly most justly, as closely related to that genus, and Kräusel and Weyland are even inclined to unite the two genera.

Baragwanatia also suggests comparison in another direction, namely, with *Barrandeina* and its relatives. Certainly, the said genus has leaves of quite another texture, which are widened distally into wedge-shaped, often dissected leaf-blades. But at least in habit there seems to be a good deal of resemblance, and also in *Barrandeina* the sporangia were situated on the upper side of sporophylls, though in larger numbers. It is interesting to note this fact, because *Barrandeina*, *Duisbergia* and others have been regarded by several authors as side branches from the base of the evolutional line of the Lycopodiales, and as representing more or less futile attempts by the microphyllous plants to form large leaves.

This idea, however, at present rests on a quite insufficient basis such as the further accumulation of facts is necessary to clear up one plane nature of the leaves of *Barrandeina* and its consorts, and. These relation to *Drepanophycus-Baragwanatia*. But at all events Isabel appears natural to attribute some importance to the intermediate position of *Drepanophycus* between the psilophytes and the lycopods.

Finally, mention must be made of *Protoplepidodendron*.

The structure of the Middle Devonian *P. scharyanum* has been cleared up by Kräusel and Weyland on German and Bohemian material (30, 31). This species occurs in Scotland and probably in Australia, while the American species often referred to the same genus is something quite different. The plant is supposed to have resembled a modern *Lycopodium*, except that it was somewhat larger; the axes might have measured a couple of centimeters across and contained a triangular protoxylem. They were densely clad with leaves which differed from those of *Lycopodium* in being forked near the tip. On the fertile axes the leaves were more distant and bore a sporangium on the upper side (Fig. 2c).

That this plant is related to the lycopods is generally accepted, but opinions differ as to its interpretation. W. Zimmermann, to whom the lycopodiaceous microphyll is developed from a dichotomously divided sterile telome and not from evaginations, regards the forked leaves as intermediate stages in the process of reduction. This view would give *Protoplepidodendron* an important place at the base of the ancestral line of the lycopods, but the question could scarcely be regarded as settled so far. Most paleobotanists would probably prefer to regard the genus as more distantly related to the modern group.

FERN-LIKE PLANTS. In connection with the reference to Lignier's view it was mentioned above that plants like *Psilophyton goldschmidtii* (and *Astroxylo*), with their naked flattened lateral shoots, may be regarded, at least morphologically, as an approach to macrophyllous plants. But they still represent, at most, only an initial stage.

Among the other early Devonian plants there were a considerable number which must have had a decidedly fern-like appearance. Best known among them, and indeed one of the best known of Devonian plants, is *Anaeurophyton germanicum*, described from the Rhenish Devonian by Kräusel and Weyland (25, 26, 27).

and really *m'on* had the habit of a tree-fern, with a stout main axis, the which is almost completely cleared up. There was no pith as *indica'* strand of primary xylem of trilobed outline, the protoxylem sporangiate in the ends of the lobes. This strand was surrounded by within.y xylem consisting of radial rows of pitted tracheides and un-justif. wood rays of very considerable height (60 cells and more). The lyc. organs resembled large fern leaves but their vascular bundle was *ilt* like that of the axis, and in consequence thereof the branching seems *a'* have taken place in three directions, not in one plane only. There was no real lamina, but the ultimate ramifications were simple, linear, recurved, and had no vascular bundle. The sporangia were borne in dense clusters on fertile "leaves" resembling the sterile ones; they were oval and dehisced longitudinally without any elaborate opening mechanism. Spores were tetrahedral, with spines ending in grapnel-like hooks. The authors (Kräusel and Weyland) reckon with the possibility that there might have been two kinds of sporangia, of which the microsporangia alone are well known; the uncertainty on this point is very regrettable.

The close relation of *Aneurophyton* to *Eospermatopteris*, the famous "Gilboa tree" from the Catskills in the State of New York, has been demonstrated by the said authors (32). Perhaps there is a generic identity. There is also a striking resemblance to *Rhacophytum condrusorum* of the Upper Devonian of Belgium as well as to other Upper Devonian plants, for example, from Bear Island. These plants seem, however, to have had dorsiventral leaves expanded in one plane only, and on account of this fact and the difference in the fertile parts, the resemblance is probably more superficial and casual than might be supposed at first sight. The structure of the wood of *Aneurophyton* in many respects corresponds to that of *Palaeopitys*, which is further mentioned below.

The importance of *Aneurophyton* lies in its combination of various characters. The anatomy of the stem and the position and structure of the sporangia show primitive features as well as distinct hints toward more advanced types, and from a morphological view it is of the utmost interest. Its habit must have been rather fern-like, but its "leaves" had the anatomy and probably also the branching of an axis, and there was scarcely any trace of a lamina, and no "webbing." It affords an excellent example of the transition from axial to foliar macrophyllous organs. Opinions may diverge, however, as to whether it represents a tentative but unsuccessful experiment in that direction, not leading to any further phylogenetic results, or whether it really lies more or less directly in the line leading to the later ferns or pteridosperms.

It is interesting to note that in early ferns, such as the Coenopterideae, the leaves were branched in more than one plane and comparatively slightly different from the axial organs. These plants, however, have been treated fully in a paper by Lady Isabel Browne (6), and the discussion will not be repeated here, the more so because we do not know whether the nearest relatives of *Aneurophyton* should be sought among the pteridosperms, or the true ferns, or among both of them,—if among any. Arnold (4) thinks that the pteridosperms and the Cordaitales may have sprung from a common source comparable with *Aneurophyton*, *Palaeopitys* and other plants with similar types of wood. Before this question can be solved very much more information is needed, especially with regard to the reproductive organs of the plants in question. As mentioned, it is possible that some of them were heterosporous, but this has not been proved, and as to seeds, none has yet been definitely demonstrated from rocks older than the upper part of the Upper Devonian. From the latter we have *Xenotheca devonica* (2) from North Devon, a problematic cupule-like fossil, the parent plant of which is entirely unknown. There are also the seed-like structures which Arnold (3) recently described from northern Pennsylvania, where they occurred together with *Archaeopteris*, an association which involves the possibility, discussed by the said author, that they belong together. It would, however, be very surprising if this were really so in view of the many occurrences of *Archaeopteris* in other places, where innumerable leaves and sporangia are abundant but where no seed has ever been found nor any organ which might be supposed to have been seed-bearing.

We may, therefore, say that *Aneurophyton*, like so many other Devonian plants, facilitates the understanding of the origin of the macrophyllous leaf, but that its affinity to later macrophyllous plants is doubtful.

There are some other Middle Devonian plants which also show an approach to the macrophyllous stage, but which are less advanced:

Protopteridium (Fig. 3 b), as defined by Kräusel and Weyland (31), comprises four species from Bohemia, the Rhineland and Scotland. The habit of the entire plant and the morphology of its branch-systems have not yet been finally clarified. But it is certain that it had *Hostimella*-like axes, bearing lateral branches alternately in one plane. Some of the latter divided repeatedly, at least in the first divisions by dichotomy, so as to

form sub-pinnate branch-systems of a frond-like appearance; even the ultimate tips seem, however, to have been terete, at least in the Bohemian *P. hostimense*. Young branch-systems of this kind and the upper part of older ones were incurved. Other branches of the same type of ramification bore numerous linear sporangia, containing, as demonstrated by Lang in *P. thomsoni*, large spores surrounded by a broad wing. According to Kräusel and Weyland, these axes, at least in *P. hostimense*, were connected with branch-systems of ordinary *Hostimella*-type, without the frond-like parts.

The habit of this plant is in many respects just as fern-like as *Aneurophyton*, perhaps even more so, on account of the dorsiventral branching. On the other hand, although it would be impossible to include it in the Psilophytale, there is no very wide step to that group. There is one particular point which is of some interest in this connection. In Bohemia, *Protopteridium* occurs

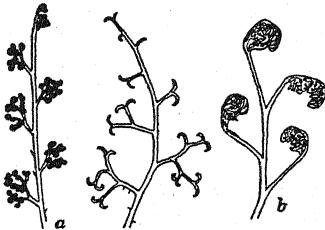


FIG. 3.—*a*: *Dawsonites ellenaе*, two shoots, about natural size; *b*: *Protopteridium hostimense*, young sporangiferous shoot, about half nat. size. (*a*, original; *b*, after Kräusel & Weyland.)

together with numerous branch-systems which form the prototypes of *Hostimella*: Dichotomously or sometimes laterally divided spineless axes, sometimes with the characteristic axillary structures which have been called "buds" (and which may perhaps really have been a kind of bud, to judge from the interesting specimen, with a branch in this place, which was described from Australia by Lang and Cookson 1930 [37, 8],—compare also *Gosslingia*). Although no instance of direct organic connection has been found, Kräusel and Weyland, in their reconstruction of the plant, have combined these *Hostimella*-branches with *Protopteridium*. Now *Hostimella*-branches with "buds" are rather widely spread in the Middle Devonian (Germany, Great Britain, Norway, Spitsbergen, France, Australia), occurring in places where it is scarcely possible to believe that they formed part of any species of *Protopteridium*. In most cases, nothing or very little is known about the

plants to which they belonged, except that, as a rule, it has been accepted that they were psilophytes. In the Rhenish Middle Devonian, such branches have been combined by Kräusel and Weyland with *Asteroxylon elberfeldense*. These axillary "buds" are very characteristic and peculiar structures, and it would be surprising if they had come into existence by parallel development independently in several groups of plants. It is much more likely that they are homologous and bear witness to a real relationship. If this is the case and if the combinations of bud-bearing hostimellas with *Protopteridium* and with *Asteroxylon*, respectively, are correct, it would be unavoidable to attribute some phylogenetic importance to them.

Dawsonites Ellenaæ (Fig. 3a) (18), from the Middle Devonian of Western Norway, is also of some interest in this connection.

Only fragments were found but they comprised both fertile and sterile parts. The former had sporangia (more correct: flattened, round or reniform bodies supposed to be sporangia), borne in clusters on short, repeatedly divided, lateral branches in (?) spiral arrangement along a common axis which was sparingly armed with short spines. In the sterile branch-systems the side branches, which seem to have been bilateral instead of spiral, were repeatedly divided, either dichotomously or monopodially. The ultimate tips were recurved. Spines similar to those on the fertile parts were scattered on the surface.

That this plant is a psilophyte is very probable, and it was therefore placed in the artificial genus of *Dawsonites*, comprising psilophytalean sporangia of unknown affinity. But it is really much too well characterised for this, and will have to be transferred to a genus of its own. There is no trace in it of the pinnate leaves of *Protopteridium*, but the lateral clusters of sporangia recall those of that plant, and the sterile branch-systems resemble initial macrophyllous leaves, as do those of *Psilophyton goldschmidii*. Whether there is any real affinity to *Protopteridium* must be left to the future to decide.

Quite recently, some other plants have been described which are of importance when discussing the relation between psilophytes and Pteropsida: *Yarravia* and *Hedeia*, both of them described by Lang and Cookson, from the Silurian of Australia; their age alone was sufficient to cause sensation.

Yarravia (Fig. 4a) (8, 38), of which two species were distinguished, consists of a stalk terminated by a small number (5-6) of linear-oval

sporangia which were parallel to each other and in all probability coherent, leaving an open central space between them; the tips alone were free. The whole fructification was about 1 cm. long. The reconstruction was given with cautious reserve by the authors, and the interpretation could not be confirmed by the demonstration of spores; but in all probability it is correct.

Unfortunately, nothing is known of the plant of which *Yarravia* formed a part, but there is good reason to believe that it had its place among the Psilophytale. In surface view the fossil very much resembles *Sporogonites*, but it is really something quite different. It is a typical apical "synangium," a group of connate

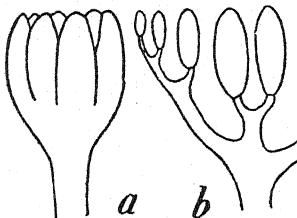


FIG. 4.—*a*: *Yarravia*; *b*: *Hedeia*. (Based upon photographs by Lang & Cookson.)

sporangia, very similar to the reproductive organs of *Whittleseyinae*, which Professor Halle has recently cleared up and which he supposed represented, in all probability, the micro-sporangia of pteridosperms.

The morphological and systematical interpretation of *Yarravia* was corroborated through the later discovery of *Hedeia* (Fig. 4b) (8):

The following diagnosis was given by Miss Cookson: "Fertile branch-systems, the main axis bearing secondary branch-systems terminally. Large elongate-oval sporangia terminate the sub-divisions of the secondary branches, all their tips coming to the same level in the corymb-like branch-system. Secondary branches may either subdivide dichotomously, the terminal sporangia all being the same length, or the sporangia may be borne on the inner side of the branch-system, those centrally placed being longer than the more distal. In some cases, the ultimate division of the secondary branch-system is sterile."—The sporangia were 5–9 mm. long and 1–2 mm. broad. It is regarded as probable that the branches, at least originally, were radially arranged.

Hedeia is a complex structure, if compared with most psilophytes. But it is more simple than *Yarravia*, and makes it more easily conceivable how such synangia have come into existence. It is not necessary, however, to suppose that during their development they passed through a stage like that of *Hedeia*.

Halle, when discussing the synangia of *Whittleseyinae* (13, 57), pointed out the possibility that they might have sprung from a psilophytalean ancestor with a terminal umbel-like cluster of sporangia, as sometimes found. If such sporangia arranged themselves in a cyclic manner and became connected with each other so as to form a hollow cylinder, this process would correspond to the lateral fusion (webbing) of telomes forming a leaf-blade. *Hedeia* is considerably more complex than the assumed ancestor (which really is not merely hypothetical), and may perhaps be more distant from the line of ancestry of *Yarravia* than appears likely at first sight.

However this may be, and whether the Silurian and the younger synangia of this type phylogenetically belong to one group or not, the former possess great interest in showing the abundance of plant forms in the Devonian and earlier floras, and how structures which have been supposed to belong to later times are really foreshadowed by the psilophytes and their relatives and contemporaries.

Finally, it may be of interest to mention a plant of which only the anatomy of the stems is known, *viz.*, *Schizopodium Davidi* (Fig. 1b) (15), from the Middle Devonian of Queensland.

Of this plant only a number of leafless, dichotomously branched axes, 3-25 mm. wide, are known densely packed together in a small block of chert which had preserved them to perfection. It had a star-shaped stipe with protoxylem near the ends of the rays, very much like that of *Asteroxylon*; but it sometimes disintegrated into separate strands of xylem, and the outer part of the xylem had an appearance like that of secondary wood, the tracheides being arranged in radial rows. There was no cambium, however, and the wood can not be described as truly secondary. The tracheides had multiseriate bordered pits.

The structure of the wood, and above all the pitting of the tracheides, resembles that of *Palaeopitys milleri* from the Middle Devonian of Scotland; but as nothing is known about the affinities of the latter (which also differed in the structure of the inner wood), this fact does not give much help as to their systematical position. *Schizopodium*, however, is of the greatest interest, not only on account of its wonderful preservation, which gives great expectations as to further collecting, but also because there is good reason to regard it as a further development from a type like *Asteroxylon*, possibly towards such "macrophyllous" plants as *Aneurophyton*, and further towards the pteridosperms.

Summing up the preceding pages, one may state that the floras particularly of the Middle Devonian comprise a number of plant forms which show distinct affinities to the Psilophytale and at the same time give reason to suppose that this group is connected with the later macrophyllous plants by a series of intermediate forms.

ARTICULATAE. Of articulate plants there are several representatives in the Devonian floras. *Sphenophyllum* has been reported in various species from the Upper Devonian of Bear Island, Belgium and (?) France; and *Pseudobornia*, which probably is remotely related to the other Articulatae, is known from Bear Island only. Of greater interest on the present occasion are the Middle Devonian genera *Calamophyton* and *Hyenia*, which have been united by Kräusel and Weyland under the name of Protoarticulatae. The former genus, known only from the Rhenish Devonian, is the most advanced type and may probably have been derived from a more primitive one resembling *Hyenia*. We shall, therefore, confine ourselves to a few words on the latter.

Hyenia (Fig. 5) has been found in some closely related species in Norway (Nathorst gave it its name from the locality Hyen), Spitzbergen, the Rhine Land and Belgium, and, as is the case with so many other Middle Devonian plants, its morphology has been cleared up by Kräusel and Wey-

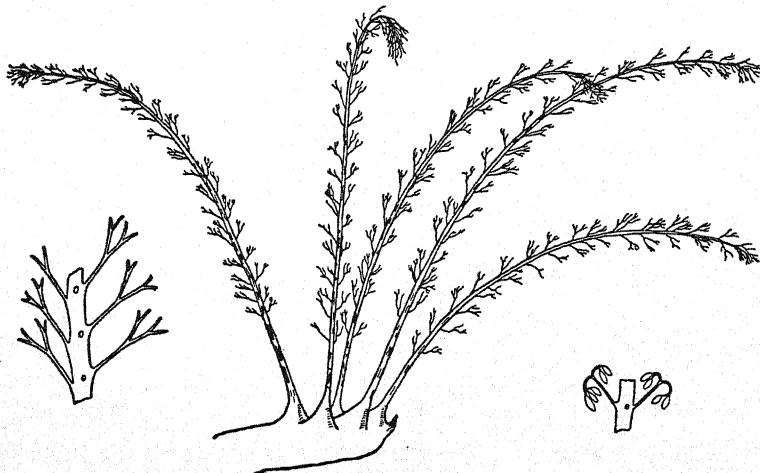


FIG. 5.—*Hyenia*. (Chiefly based upon drawings by Kräusel & Weyland.)

land on the basis of German specimens. The plant had a horizontal rhizome bearing aerial shoots which were only sparingly branched or not at all. The leaves were rather small and divided in more or less regular dichotomy into filiform segments; they were arranged in a semi-vermicillate manner, the whorls in some parts of the plant being quite regular, in other parts giving way to a spiral arrangement. In the upper portion the stem might have been fertile, the leaves in that case dividing dichotomously a few times and at short intervals, and bearing a sporangium on each tip; in the fertile region the arrangement was regularly vermicillate.

Hyenia is interesting in many respects. The vermicillation seems to denote an approach toward the Articulatae, and Kräusel and Weyland, certainly with good reason, have regarded the Proto-articulatae as phylogenetically connected with the early Calamariaceae.

From a morphological point of view it is of interest to recall the well-known fact that the oldest species of *Sphenophyllum* had dissected leaves (or rather, leaves which had not yet formed broad wedge-shaped blades), resembling those of *Hyenia*. There is also a most striking similarity in form between the latter (particularly in the case of *H. elegans*) and the pinnules of some species of *Archaeopteris*, such as *A. fissilis*. The phylogenetical bearing of this fact is difficult to judge at present; but it is worth while to bear in mind Lignier's words, formed before *Hyenia* was known, that it is to *Archaeopteris* that the sphenophyllums seem to present the closest affinity (39, 283). Since his time the question has become more complex, but the idea has scarcely lost its value.

The origin of *Hyenia* is still hidden in darkness. Like the true Articulatae it is usually termed "microphyllous." But the same designation is generally employed in the case of the lycopods as well, although it has not been proved that the leaves in the two cases are of the same nature; probably they are not. If the leaves of *Hyenia* are regarded as altered axial organs, the terminal position of the sporangia on the divisions of the sporophylls is a parallel to the conditions among the psilotophytes. But the vermicillate arrangement, however variable and unsettled, and the pronounced contrast between the foliar organs and the stems, are characters not found within the latter group. Intermediate stages may easily be imagined, but they have not yet been found, and until that happens the connections of the Articulatae will remain uncertain.

ORIGIN OF THE PSILOPHYTES

It is not necessary here to enter further upon the other groups of vascular plants. What has been said may suffice to demonstrate that the various groups of higher plants are represented, or at least foreshadowed, in the early Devonian floras, and their convergence toward the central group, the *Psilophytales*, is at least strong enough to show that a solution of the question of the origin of the latter will also solve that of the origin of vascular plants as a whole.

In asking for the origin of the psilophytes, one may consider separately their relationship to the mosses, and their possible algal affinities. But first another point must be touched on.

RHYNIACEAE, PRIMITIVE OR REDUCED?—Most simply organized among the psilophytes are *Rhynia* and *Hornea*, particularly the former genus. Certainly the adventitious branches, present in one of the two species of the genus but lacking in the other, denotes a kind of specialisation. But apart from this feature its organs and tissues represent the minimum of what could be expected in a vascular terrestrial plant, and the degree of differentiation is nearly as low as possible. This also applies to the fertile region. It is as ideal an illustration of an early stage in the development of higher plants as Lignier's theory could possibly picture.

From a morphological point of view, it might seem a matter of minor importance whether *Rhynia* and its relatives were really primitive plants, or were derived through a process of reduction from more highly organized ancestors. The question in itself is, however, of considerable interest; the latter view has been maintained by several botanists, and it would be in accordance with the relatively young age of these plants, the Rhynie Chert Beds belonging somewhere in the middle part of the Old Red, whereas other psilophytes are known from much older horizons.

The vegetation preserved in this chert must have been exposed to conditions of a special nature. Possibly the habitat was "a swamp beside a stream or the edge of a small lake or pond, perhaps fed by the water from a fumarole or siliceous spring" (22, 896.) To a great extent the plants must have become imbedded in the silica *in situ* before decaying, or even before falling. On comparison with recent siliceous springs it appears rather probable that the water was hot. Growing in such a habitat necessarily must have

meant a transition to a new kind of habitat, a transition which might be thought to have favoured a retrogressive development. If reduction could be proved to have taken place, one might look for the explanation in this change of environment. Conversely, however, the nature of the habitat can not be used as a proof of the correctness of the idea of reduction. The association of *Rhynia* with *Asteroxylon*, one of the most highly developed psilophytes, is also noteworthy in this connection.

If *Rhynia* had really been developed along a line of reduction from more highly organized forms, one might have expected to find some rudiments inherited from these ancestors, or some characters inconsistent with the general primitiveness. It appears impossible, however, to point out any such characters. Therefore, the idea of *Rhynia* being a reduced form does not seem to be necessary, and nothing would be gained by accepting it.

At present it will be necessary to base considerations as to the origin of the psilophytes above all upon *Rhynia* and *Hornea*.

Concerning fossil evidences of the origin of these plants, only one case may be mentioned.

As pointed out by Kidston and Lang, the sporangia of the plants under consideration are so slightly differentiated from the stem (compare the casual bifurcating of the sporangium in *Hornea*) that it is easy to imagine the next step below this, *viz.*, a thalloid plant body in which the spores developed within the very tips of the thallus branches without any transformation into a defined sporangium. Such a stage is represented by *Foerstia furcata* (Fig. 6b).

In 1888 Dawson described some peculiar plant remains from the black oil shales of the Upper Devonian of Ohio under the name of *Sporocarpum furcatum*. They were redescribed in 1923 by White and Stadnichenko (44) under the name of *Foerstia ohioensis*, and in 1924 by Kidston and Lang (24). Thanks to these redescriptions we know this plant—which will have to be called *Foerstia furcata*—in more structural details than its fragmentary state of preservation would have allowed us to hope. What is left of it looks like the tips of a thalloid plant body, consisting of coherent cellular tissue; they are a few millimeters in length, often forking, and contain resistant, probably cuticularised, spores in tetrads. The latter are scattered within the tissue at some distance from the

margin and chiefly around the apical incision. Nothing is known of the other parts of the plant, a remarkable fact in view of the enormous number of tips littered in the rock.

Summarizing the little that is known of the plant, we may regard it as a thalloid and possibly alga-like plant, probably not vascular, and with spores of such a nature that they must be supposed to have been adapted to sub-aerial conditions, but not developed within definite sporangia. The latter character makes it appear more primitive than any psilophyte, and at least in a morphological sense suggests a link between the latter and their unknown ancestors. But it could not be said to denote an approach to any familiar plant group. The form of the thallus recalls those of certain liverworts, but its nature as a sporophyte makes further comparison impossible.

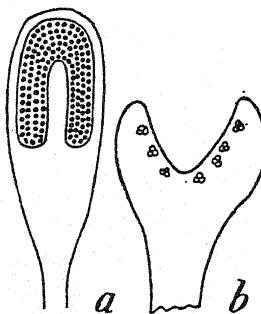


FIG. 6.—*a*: *Sporogonites*, diagrammatic longitudinal section; *b*: *Foerstia furcata*. (Not drawn to scale. Based upon drawings and photographs by: *a*, Halle, *b*, Kidston & Lang.)

Stigmophyton sturi (31) from the Middle Devonian of Bohemia is known only from a few fragments of axes, scarcely 1 cm. wide. Scattered on the surface of the fossils are small scar-like bodies, consisting of a carbonaceous matter which Kräusel and Weyland with all reserve have interpreted as possibly representing remains of spores.

The habit and affinities of *Stigmophyton* unfortunately are unknown. But at all events, if the interpretation of the "spores" is correct, it is a plant which had definite sporangia, scattered, however, within the tissue of the thallus instead of being confined to the apex. Like several other Devonian plants, it opens wide views to the imagination and strains one's expectations of the future.

PSILOPHYTALES AND BRYALES. Apart from being independent of the gametophyte as to nutrition, possessing tra-

cheides in the central strand, and being branched, however sparingly, the body of *Rhynia* or *Hornea* is not very much different from the sporophyte of a moss. A similarity still more striking is found in the isolated fructifications known as *Sporogonites* (Fig. 6a).

Sporogonites is a name introduced by Halle for a species, *S. exuberans*, which he found at Röragen in Norway, about 20 years ago. In 1930 Lang and Cookson recorded two specimens of another species, *S. chapmanni*, from Australia, and recently, Professor Halle has re-examined his Röragen material (12, 14, 37). The fossil consists of a simple stalk, straight and slender, widening terminally so as to form an oval capsule, about 6-9 mm. long, longitudinally furrowed. An important point is that the lower half of it consisted exclusively of sterile tissue. The upper part had a multi-layered wall surrounding a dome-shaped spore-sac; the central part of the latter seems to have been filled by sterile tissue, a columella, surrounded on the sides and above by the spores. All spores of one kind, about 20-25 μ . Mode of dehiscence unknown.

A capsule like this is not, in its fundamental characters, different from that of *Rhynia* or *Hornea*. There is no difficulty, from what little we know of *Sporogonites*, to suppose it to have formed part of a psilophyte, perhaps of the same family as those mentioned. But what gives it its outstanding importance is its resemblance to a moss sporogonium, on account of the great amount of sterile tissue in its basal part. Halle compared it principally with *Sphagnum*. Certainly the greater number of mosses have a calyptra, and the opening mechanism is rather complex. But no very elaborate constructions are needed to picture the missing links between the mosses, on the one side, and *Sporogonites* and the psilophytes, on the other.

It might appear natural to regard the mosses as primitive in relation to vascular plants. But there is also another possibility. We can do no better than quote Scott (42, 350), who accepts the words of Bower: "The new facts are thus seen to link the bryophytes and the pteridophytes more closely together than ever before;" and who further states: "Here we will only recall Haberlandt's opinion that the mosses were reduced forms. At that time one asked: But reduced from what? Possibly the *Rhyniaceae* may suggest an answer."

Unfortunately, the mosses themselves do not contribute much to the solution. The few which have been found as fossils (*Heptaticae* and *Musci* in the Carboniferous rocks) are too much like

the modern ones to render any phylogenetic information. The morphology of the sporophyte of the true mosses is stereotyped to a degree which is really a problem in itself: Always the same simple seta, long or short or practically absent, but never branched, except in a few teratological cases of unknown importance; never bearing any kind of emergences or the faintest trace of a leaf. And the sporogonium: Always a solitary capsule terminating the seta; it may be pendulous or erect, varying a good deal in the number of teeth and in other details which from our point of view are quite uninteresting; varying slightly, however, in such structural details that could give us some information. It is an unparalleled uniformity and conservatism, the more surprising because it characterises a group of some 12,000 species.

But there are some points of interest: (1) One of them concerns the stomata of the sporogonium of the *Musci*. The fact that they occur on the sporophyte only, and are often reduced and rudimentary, would be most easily understood if they were reminiscences from early ancestors in which the sporophyte was independent of the gametophyte as far as nutrition was concerned. (2) The sporophyte of *Anthoceros*, in contrast to that of the true *Hepaticae*, is green, long-living, growing by means of an intercalary meristematic tissue, and, like the gametophyte, possesses stomata. This long cylindrical body is not differentiated into capsule and stalk, but the spores are contained in the usually columellate cavity of the upper part, and dehiscence is carried out by a simple longitudinal splitting. No plant is known which shows structures more like those of the most primitive psilophytes, and it is easy to assume a relationship. In this connection it is interesting to note the relatively slight difference between the sporophyte and the gametophyte of *Anthoceros*, a fact giving support to the assumption of a "homologous" alternation of generations in the ancestors of the cormophytes.

But the relationship of *Anthoceros* to other mosses is uncertain and possibly rather remote (7), and, at all events, most of what may be said about these questions will remain hypothetic until we have gained some knowledge of the gametophyte of the psilophytes. It is perhaps not altogether beyond hope that our regrettable ignorance on this point will be removed one day; the wonderful preservation of the Rhynie plants and of the Australian *Schizopodium*

makes one optimistic. As to the question of the gametophyte of the psilophytes it has, on the other hand, been pointed out by several authors that it is not probable that plants like *Rhynia* had any large and well developed sexual generation; otherwise, one should have expected to find it in the Rhynie chert where certain layers were entirely filled with perfectly preserved complete specimens of one or the other species.

From the present stage of knowledge it seems probable that the mosses have risen from the same source as the psilophytes, but at a very early point.

PSILOPHYTES AND ALGAE. Though simple in habit, the thalloid psilophytes are rather complex from a histological point of view, and it is impossible to connect them with any known group of algae.

Certainly remarkable analogies may be pointed out. As to anatomy, there are, among both the brown and red algae, forms with marked differentiation of tissues, possessing assimilating parenchyma and strands made up of sclerotic elements together with conducting cells comparable to the sieve-tubes of vascular plants. That there are no tracheides or vessels is only to be expected in sub-aquatic plants with no demands for water transport.

Concerning organs of reproduction, the spores of pteridophytes are of the same nature as the tetraspores of algae; the sporangium of a psilophyte is homologous not with an algal tetrasporangium, which contains only four spores, but with a sorus of tetrasporangia. In many red algae such sori are imbedded in the tissue of the thallus, sometimes in the tips or in special small branches, called stichidia. The latter, as pointed out by Kidston and Lang in 1920 (19, 622), bear a considerable resemblance to sporangia of the *Rhyniaceae*.

However, these algae are highly specialised forms which are not likely to have given rise to an entirely new branch of the plant kingdom. This fact, and still more their mode of reproduction and their cytological and physiological properties, make it difficult to believe that any *Phaeophyceae* or *Rhodophyceae* should represent anything like the algal ancestors of the vascular plants.

If the psilophytes are descended from any group of algae of which subaquatic members are still living (not necessarily, of

course), it is the green algae, the *Euchlorophyceae*, that offer themselves for comparison in spite of all dissimilarity. We have, however, no fossil records that might help to fill the immense gap between these algae and even the most primitive terrestrial or amphibious forms among the plants mentioned above, so the question will not be further entered upon on this occasion. The algae which have been described from the Silurian and Devonian periods are interesting enough but of slight value in this connection.

Like the whole question of the origin of the psilophytes, their relation to the algae can not be expected to be fully understood before we have some knowledge of their ontogenetic life cycle. As to the fascinating theoretical considerations of the mutual effect of transmigration from sea to land, on the one hand, and the somatic development of the two alternating generations, on the other, the reader may be referred to a full treatment of the subject, with references, in the latest work by Professor Bower (5, 483).

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THE BOTANICAL REVIEW

VOL. III

DECEMBER, 1937

No. 12

WILD SPECIES-HYBRIDS IN THE PHANEROGAMS

H. H. ALLAN

Botanist to the Plant Research Bureau, New Zealand

S'il n'est pas plus possible de s'attarder dans une sorte d'hybridophobie systématique, il importe tout autant de ne pas tomber dans l' hybridomanie.
Fouillade—1932.

When Lotsy (65) cast aside the results of the heavy labours of years on phylogeny as "a product of phantastic speculations" and produced that delightful provocative small volume of 1916, he indeed threw a bombshell into the evolutionary camp. But the resultant dust has now subsided and few will be found to deny his claim that he had "cut a small peep-hole in the curtain which hides the stage on which evolution takes its course," though some will fear that it is not the real play that we glimpse, that we are still victims of 'the cave.' Whereas Robson (95) in 1928 in "an introduction to the study of evolutionary divergence in natural populations" gave scant attention to hybridism, Renner (94) in 1929 produced an impressive survey of the work done on species-hybrids, although he barely touched on hybridism in nature. And at that date a new "Focke" (45) would have required several volumes, while Ostenfeld (92) concludes that: "We arrive, therefore, at the general conclusion that species-hybrids in nature *apparently* do not play any rôle worth mentioning, as the species keep constant and the hybrids soon disappear. While this may hold good in most or at least in many cases, it is by no means always so, and the complications and aberrations from this common belief are of great interest both from a purely botanical and from a more general point of view. We are here at the beginning of a new road into an understanding of the manner in which new species arise by means of crossing." In the subsequent years enthusiasts have sought to proceed along that road; sceptics have declared that the road is blind or at least leads into a maze of bewildering and misleading tracks. So we find such contrasting treatments as that of the geneticist

Babcock (10) and the taxonomist Fernald (43). Babcock finds wild hybrids to be an important feature in *Crepis*, and that inter-specific hybridization and amphidiploidy is a fundamental process: "That this phenomenon may be of great importance in the evolution of the higher plants has been amply demonstrated by the production, through artificial hybridization, of highly fertile and constant new forms which might be expected to maintain themselves under natural conditions." Fernald, in his valuable and entertaining discussion of "Some beginnings of specific differentiation in plants," does not treat of wild hybrids at all, though he insists upon the importance of field studies of the "results of the slow processes of nature."

The present article deals with some of the work done during the last few years along one small track which anastomoses freely, it is true, but which seems likely to prove of considerable value in determining where the main road should be planned and constructed. The subject is discussed at greater length, from the New Zealand standpoint, by Allan (5).

CRITERIA OF WILD HYBRIDS

Diels (37) summed up the features to be expected in wild hybrids, emphasizing their more or less "intermediate" nature, their often "monstrous" characters, their frequently "bad" pollen and defective fruiting powers, and their irregular distribution. Later work has shown that, while these features are helpful in field work, hybrids may occasionally be quite aberrant in form without being monstrous; perfectly normal in behaviour, with good pollen and setting abundant seed. There is evidence that some good species may produce defective pollen and show chromosome aberrations, and that hybrids may produce good pollen and show regular reduction divisions. Ekman (39) found that in *Draba*, crosses between species with the same chromosome number may be sterile. Huskens (53) has pointed out that there is no one satisfactory and final criterion of hybridity, and that "arguments based on any one of the supposed criteria above mentioned must lack general validity." The decisive test is experimental evidence, and even that has its limitations, for nature may have succeeded where man fails, just as man has accomplished what nature has apparently not even tried. Obviously, too, the matter is influenced by the individual worker's

conception of what a species is. Thus, what Zamelis (106) in his striking work on *Pulsatilla* treats as subspecies of *P. patens*, are considered by some taxonomists to be "good" species. It is difficult, also, to avoid entirely the danger of one's views being over-coloured by one's own experiences. It will not be disputed, however, that there are now known very many wild hybrids between what not even the hardiest of lumpers would refuse to call distinct species. It is also accepted in general that wild hybrids *can*, in large measure, be recognized in the field. Mistakes, as Ullmann (102) remarks, are inevitable. But he who risks no mistakes makes no discovery, and—*Du choc des opinions jaillit la vérité!* While, then, the field observer will rely mostly upon the criteria indicated by Cockayne (23) that the alleged hybrids should be more or less intermediate or show a graduated series linking one parent with the other, will generally be found in proximity with the parents, and if fertile, will produce a more or less polymorphic progeny, he has to recognize that all these may fail him and that repeated studies are necessary for definite conclusions.

The results of synthetic studies by H. Nilsson (85, 86, 87) show the need for great caution in interpreting field-evidence. For *Salix laurina*, for example, various origins have been proposed, almost always with *S. phylicifolia* as one parent and *S. cinerea* as the most favoured second parent. But Nilsson shows that this cross does not produce a *laurina* form, which unexpectedly turns up in the progeny of *S. caprea* \times *viminalis*. Allan (5) discusses "mimics" and "doubles," *i.e.*, forms of the same or of different rank closely resembling one another. Thus *Celmisia Armstrongii*, *C. Lyallii* and *C. Petriei* are three distinct species, yet with a general resemblance in several features. Each hybridizes with the very different *C. coriacea*. Apparently, the hybrids are sterile, and as herbarium specimens they are so similar that they could easily pass muster as belonging to a single slightly variable species. This has actually happened with *C. linearis* and *C. compacta*, these names being based on mimicking specimens of quite different hybrid origin. In *Coriaria* certain known hybrid forms can hardly be distinguished morphologically from true-breeding forms from distant localities. A sound treatment of these and other genera will be arrived at only by a combined attack using taxonomical, ecological and genetic

methods. Such work would contribute much to the species question.

PAUCIFORM AND MULTIFORM HYBRIDS

Allan (5) roughly classified 330 hybrid groups in the New Zealand flora as uniform (showing no diversity, 4), pauciform (showing comparatively little diversity, 62), and multiform (showing great diversity, 264). Many of the uniform and pauciform groups, in all countries, are probably more or less sterile first generation plants, with some true-breeding amphidiploids. Modern floras are giving increasing attention to wild hybrid groups, as, for example, that of Lindman (63). Of the very numerous accounts of individual hybrids or of small groups the following are representative: Bornmüller (12) *Verbascum*; Carse (16) *Cassinia*, *Coprosma*, *Scirpus*; Cheel (17) accepting hybridism in *Acacia*; Cuatrecasas (28) *Rosa*; Faegri (40) *Symphytum*; Hübl (55) *Carex*, *Centaurea*, *Pimpinella*; Holmberg (52) *Hypochaeris*, showing one per cent. normal pollen and reduced seed production; Jansen and Wachter (56) *Puccinellia*; Mayer (73) *Rosa*; McLuckie (74) *Grevillea*; Parker (93) *Dipterocarpus*; Sherff (97) *Dubautia*; Troitskii (99) *Agropyron*.

FERTILITY OF SPECIES-HYBRIDS

Wild species-hybrids show a great range of fertility. Fernald (41) mentions six wild interspecific hybrids in the wind-pollinated species of *Eupotamogeton*, of which he remarks: "In these cases, as in the natural hybrids in numerous other genera, the sterile hybrid combines the obvious characters of its reputed or demonstrated parents, and it occurs with or in close proximity to them." He critically examines and rejects several reputed hybrids accepted by Hagström (48), pointing out also that sterility *per se* is no evidence of hybridism in this genus which possesses many sterile species with marked powers of vegetative reproduction. Fernald (42) records sterile hybrids in *Carex* and *Juncus*, but recognizes that sterility is only negative evidence of hybridism. F. Nilsson (82) crossed the closely related *Bromus hordeaceus* and *B. mollis* (often united by a polymorphic series of forms in nature) and found the progeny highly fertile with normal pollen. Allard (6) discusses several oak hybrids, wind pollination being general in the genus, stating that "hybrid trees are always sporadic in their occurrence

and never common in any locality." Plants from acorns of *Quercus Saulii* (reputedly *Q. montana* \times *alba*) showed a dominance of the *montana* parent but considerable polymorphy. Marsden-Jones and Turrill (71) collected seed from a highly fertile hybrid between *Silene vulgaris* and *S. maritima* (one plant only of *S. vulgaris* being noted near the hybrids). They discuss in detail the 69 plants secured, and conclude that the result indicates "how the products of hybridization can be absorbed into the parent species which dominates in numbers of individuals in the immediate neighbourhood," and how "chance and infrequent crossings can increase polymorphy and heterozygosity within a cross-pollinated population of what would normally be regarded as a species." Allan (4) raised 26 plants from five berries of an intermediate hybrid, *Myrtus bullata* \times *obcordata*. The mature plants show considerable polymorphy, bridging the species, one being morphologically indistinguishable from pure-blooded *M. obcordata*. Allan (3) produced a polymorphic series of second generation plants from the artificial cross *Coprosma propinqua* \times *robusta* (the parents widely different in many details), matching swarms found in nature. This generation is highly fertile and shows a remarkable range of fruit colour. Hill (50) obtained diverse progenies from seed of hybrid *Gaultheriae* collected by himself in New Zealand, and stated that most of the hybrid *Gaultheriae* produce viable seed. Oliver (90) remarks that "The proof that *Coprosma Kirkii* is a hybrid and that *C. repens* is one of the parents was unwittingly settled many years ago by Bishop Williams, who sowed the seed of specimens of *C. Kirkii* from Portland Island. The resultant plant bore small ovate leaves very like those of *C. repens* but smaller." The proof is hardly adequate! In a dioecious species the female may well show hybrid progeny, without itself being a hybrid.

CONSTANT SPECIES-HYBRIDS

The experimental evidence for the origin of constant new forms, equivalent to the species of the taxonomist, by means of hybridization, grows imposing, but evidence from wild hybrids is still meagre, and opens up a little-touched field for cytological work. Frankel and Hair* have studied the cytology of a number of species of

* Frankel, O. H., and Hair, J. B. Studies on the cytology, genetics, and taxonomy of New Zealand *Hebe* and *Veronica* (Part I). N. 2, Jour. Sci. & Tech. 18: 669-687. 1937.

Hebe, and have made successful crosses in a few species. They find pollen sterility to be common in some species.

Frankel (unpublished) finds polyploidy to be frequent, and considers that the origin of the polyploids is probably due to hybridization, and subsequent chromosome doubling, among related forms, and not to autoploidy.

Keck (58) considers *Argyroxiphium kai* probably an allopolyploid species arising from the hybridization of *A. caligni* and *A. grayanum*. Keck (57), from herbarium and field studies, concludes that his *Penstemon neotericus* "has arisen through hybridization between *laetus* and *azureus*, but that it no longer constitutes a hybrid population, having become a stable species over a considerable territory not now occupied by the supposed ancestral species. This invasion of a district not occupied by *laetus* and *azureus* has prevented back-crossing with these species except along the borders of its range where it meets them and this fact has doubtless aided in the establishment of a species uniform in its characters over large portions of its distribution." Clausen (22), after cytological examination, concludes "there is strong evidence for the theory of the hybrid origin of *Penstemon neotericus*, and it must have arisen by amphidiploidy." Clausen refers also to his own work on *Viola arvensis* (20, 21) as a further example of a stable species produced by hybridization. Müntzing (77), crossing *Galeopsis pubescens* and *G. speciosa*, obtained a triploid and highly sterile plant in the second generation, back-crossed this with *pubescens* and obtained one seed. This developed into a tetraploid plant morphologically identical with *G. tetrahit*, showing normal reduction divisions and good fertility. H. Nilsson (87) crossed *Salix viminalis* and *caprea*, producing a form "neocinerea" equivalent in all details to *S. cinerea*, which he suggests arose by natural crossing. Huskins (54) found *Spartina Townsendii* to have the chromosome number $2n = 126$ (the alleged parents having: *alterniflora* 70, and *stricta* 56), and states: "The importance of interspecific hybridization followed by chromosome doubling as a method of plant evolution is now generally admitted. Some authors, however, are still hesitant in their recognition of its significance, since the new species produced experimentally by this method have not yet been tested for survival under natural conditions. The conditions under which *S. Townsendii* arose were in effect so close to experimental conditions as to

convince numerous eminent botanists that it must be a hybrid, even in the days when the idea of evolution by hybridization was in general disfavour. The cytological evidence seems to furnish the final confirmation. The survival value of *S. Townsendii* is undoubtedly. It has almost completely eliminated its parent species wherever it has been in contact with them. It seems justifiable to regard it as a clear-cut example of the evolutionary significance of allopolyploidy in plants." But the amazing spread of *S. Townsendii*, "overwhelming its parents," has been due to its vegetative vigour, and Chevalier (18, 19) suggests another origin. Of specimens collected on Long Island, New York, he remarks, "Le *Townsendii* se rapproche excessivement de ces deux échantillons," and considers in general that *S. Townsendii* well fits into the *S. glabra-pilosa-alterniflora* complex, and that its hybrid origin with *stricta* as a parent is unproved. If it be a hybrid its parents may well be found in the species of the above complex. F. Nilsson (84), using material derived partly from wild hybrids and partly from spontaneous crosses in breeding material of *Festuca arundinacea* \times *gigantea*, which are highly sterile, produced two progeny plants, one being an amphiploid with considerable normal pollen, but with low seed-setting powers. Kostoff* secured two dwarf amphidioploids in *Nicotiana* crosses. One set seed after selfing, but no improvement of stature could be obtained in the offspring. He concludes from his studies that: "The production of dwarf amphidioploids with a lower vitality than the parental species, the triploids, and the majority of the chromosomal aberrants, indicates that amphidiploids, or tetraploids in general, are not always giants and that polyploidy is a limited factor in evolution.

Too great an increase of the chromosome number lowers the vitality of the organism. In reality, we know very few plants in nature with 200 chromosomes or more, and these plants are not 'giants' in comparison to other species of the same genus having smaller chromosome numbers."

HYBRIDISM AMONG NATURALIZED PLANTS

Blom (11) records over twenty hybrids among the adventive flora of Sweden, noting pollen sterility in some cases. An interest-

* Kostoff, D. Polyploid hybrids *Nicotiana rustica* var. *texana* L. *Nicotiana glauca* Grah. Bull. App. Bot. U. S. S. R., Series 2, No. 9: 153-162. 1935.

ing example is the crossing of *Chenopodium album* with the Japanese *C. virgatum*. Allan (2) records over thirty supposed hybrids, most of which have been confirmed by later studies, among the naturalized plants of New Zealand, and includes various hybrids between the Australian *Acaena ovina* and indigenous species. In most, the hybrid forms were intermediate, the individual plants showing little diversity. Man's interference has often increased the opportunities for hybridism not only among indigenous species but between these and aliens, and has also brought together species widely separated in nature. Here lies a little-exploited field for study, which *inter alia* may cause revision of current ideas concerning "ecospecies" and "commiscua." Some observations for New Zealand are recorded by Allan (5). Aamodt, Johnson and Manson (1), from a study of natural and artificial hybrids, concluded that fatuoids ("false wild oats" in cultivated crops) usually arise from natural crossings, though they do not rule out the possibility that fatuoids also occur as a result of gene-mutations or of chromosome aberrations.

WILD-HYBRID POPULATIONS

Camus (14, 15), Cugnac (29, 30, 31, 32) and Cugnac and Camus (33, 34) have studied wild hybrids within the *Eubromus* group of *Bromus*. Several pauciform hybrids are described "des caractères intermédiaires entre ceux des deux parents," and some more polymorphic groups. Thus, from the characters of the forms, together with their distribution in the field, they state that "la présence constante, en relations intimes avec *B. Gussonii*, de *B. rigidus*, d'une part, et de *B. sterilis* d'autre part, nous a donné l'idée que le *B. Gussonii* pourrait bien n'être que la désignation globale de l'ensemble des hybrides, à disjonction polymorphe, résultant du croisement *B. rigidus* \times *sterilis*." A striking feature, among the many discussed, is that *B. rigidus* has three, *B. sterilis* two, and *B. Gussonii* "tantôt deux, tantôt trois, et cela non seulement dans une même panicule, mais très fréquemment dans un même épillet." To the objections of Fouillade (not seen by the present writer) he replies that that worker was dealing with material of unproven purity, and that, "La cleistogamie, ou tout au moins l'auto-fécondation naturelle, est certainement possible, mais elle n'a point le caractère obligatoire ou presque obligatoire dont M. Fouillade tire

argument pour conclure à la 'quasi-impossibilité des fécondations croisées chez *B. maximus*.' He finds that cleistogamy is not at all obligatory in *B. sterilis*. It may be noted that *B. Gussonii* is abundantly naturalized in New Zealand, *B. sterilis* only less so, and that *B. rigidus* has not been met with. Both species set abundant seed and show no signs of segregation of characters towards *B. rigidus*. Definite hybrids have not been noted, but in both the stamens may be three or two (prevailingly three in *Gussonii* and two in *sterilis*). Fouillade (46) studied various populations of *Agrostis alba*, *A. vulgaris* and *A. castellana*. After a detailed account he states, "Les recherches que j'ai poursuivies [for twenty years] m'ont amené à la conviction que l'enchaînement continu d'individus intermédiaires qui relie entre elles les trois espèces précitées a pour facteur principal sinon unique l'hybridité." The supposed hybrids showed up to sixty per cent. bad pollen, the parents "un nombre infime de grains mal venus." He considers there is good evidence of the occurrence of ternary hybrids—e.g., *A. (alba × vulgaris) × castellana*—and "hybrides d'hybrides"—e.g., *A. (alba × vulgaris) × (alba × castellana)*. Malte (68) states: "In the genus *Agrostis* several hybrids are well-known, and in all cases their hybrid nature manifests itself by a very high degree of sterility," and refers to Murbeck's earlier work on Swedish forms in support of his views. His field work leads him to conclude that natural hybrids are rare. Unpublished work by V. D. Zotov and the writer, in New Zealand, tends rather to support Fouillade than Malte, but there is evidence that within each species (linneon) there are a number of jordanons, and no decisive conclusions can be arrived at in such groups without genetical investigation.

Anderson and Woodson (7) give numerous natural hybrids in *Tradescantia*, while Anderson and Sax (8) give details of a hybrid population between *T. caniculata* and *T. humilis*. Samples were collected and subjected to analysis over a period of three years. They state that: "The evidence may be summarized by saying that the plants thought to be *T. caniculata* and *T. humilis* were cytologically regular and agreed morphologically with other representatives of those two species collected from nearby colonies where hybridization was not taking place. The suspected hybrids presented a number of cytological abnormalities often associated with hybridization, namely, high percentages of sterile pollen, micro-

nuclei in the tetrads, etc. Certain of the hybrids were, furthermore, morphologically identical with the artificial hybrids raised between the same two species, and the whole case may be taken as proved." They find the barriers preventing frequent hybridization in *Tradescantia* to be (1) incompatible chromosome numbers, (2) time of blooming, (3) habitat requirements.

Weimarck* monographed *Clifforia*, recognizing 78 species, grouped into 8 sections. He describes 6 hybrids, distributed among 4 sections. He also admits one inter-section hybrid. These are all represented by single or very few specimens, and all appear to be sterile, with mostly shrivelled pollen and deformed receptacles.

POLYMORPHIC GROUPS

Levyns (61, 62), in her revision of *Lobostemon* and *Elytropappus*, found field evidence of species-hybrids. In *L. glaucophyllus* \times *laevigatus* some populations showed a bewildering variety of types. In *Elytropappus* hybridism is frequent within the species of two of her three sections, but no inter-section hybrids were noted, even where the species grow massed together. Melderis (75), in an important paper on *Erythraea*, describes in detail hybrids between various species, discussing also species to which he ascribes a hybridogenous origin. Numerous artificial crosses were made supporting the field evidence. Oliver (89) accepts 32 species of *Dracophyllum*, and 9 hybrids—all in the subgenus *Ozothamnus*. He gives short diagnoses of these, but recognizes that "a series of hybrids between any two species may include forms grading into both parents." Oliver (90) discusses at some length the 19 species-hybrids of which he has seen specimens in *Coprosma*, and finds that 6 occur in the same species group, 4 in allied groups, 2 in distant groups of the same section, and 7 in different sections. He considers: "That hybrids are almost invariably found in association with both parents proves that either they soon by intercrossing eliminate the unstable heterozygous characters or their power of producing fertile offspring lasts only a few generations." Burtt and Hill (13), the latter having made many field observations, find that all the New Zealand species of *Gaultheria* hybridize, in many combinations. These are discussed and their general polymorphy noted. They note that though *G. oppositifolia*, the only member

* Weimarck, H. Monograph of the genus *Clifforia*. Lund. 1934.

of the genus to have opposite leaves, definitely hybridizes with other species, it is not as yet certain that any departure from a strictly opposite-leaved arrangement is always due to hybridization, as seedlings of apparently pure origin may show a tendency to alternate leaves. They give some evidence to show that in more than one species the corolla may be pubescent or glabrous within, without there being any evidence of interspecific hybridization. Varietal forms, it may be noted, in *Gaultheria* are not always easy to distinguish from hybrid forms. This is true for other genera and needs careful consideration in field work. Cockayne and Atkinson (27) give a fully illustrated account of the great hybrid polymorphy in *Nothofagus*, involving all the species but one and probably including ternary hybrids. As characters not known in the parents, they mention longitudinal ridges on the leaves, and bullate leaves. These, however, have been noted in but few specimens.

SOMATIC SEGREGATION

Allan (4, 5) gives instances of somatic segregation in individual hybrid plants, including an intermediate form of *Nothofagus fusca* × *Solanderi* that had developed a trunk-like branch from near the base with pure *fusca* leaf characters. Cockayne and Allan (25) mention "a specimen of *Olearia* bearing leaves, some of which have the pure white tomentum of *O. virgata*, others the rusty tomentum of *O. divaricata* [a closely allied species of similar leaf size and shape], and others again of intermediate character." They suggest that partial segregation of characters may be the cause of much of the minor diversity shown in many individual hybrid plants. Examples are known in *Coprosma* of plants bearing fruit of different colours. Burtt and Hill (13), speaking of certain plants of the group *Gaultheria rupestris* var. *parviflora* × *G. depressa*, state that "The fruits are of particular interest since in some the calyx segments are wholly fleshy, in others the base may be fleshy and the tips or other half dry, while in a few flowers one or two segments are fleshy and the others quite unswollen." Trelease (98) remarks on a specimen of *Quercus Bebbiana*, a presumptive cross between the bur and the white oak, with some short acorns showing "the familiar dull downiness of a bur oak acorn," and others with "the glossy elongated acorns and shallow cups of the white oak, but with the cups somewhat fringed as they never are in a pure white oak."

REGIONAL SURVEYS

Lotsy and Goddijn (67) noted in South Africa 43 hybrids in 13 families, including 5 in *Aloe*, 7 in *Cotyledon* and 13 in *Pelargonium*. The hybrids in *Cotyledon* and *Euphorbia* are fully described and illustrated, and *Cotyledon* is considered an exceptionally good genus for experiment. A special study was made of the swarms found in *C. coruscans* \times *teretifolia*, the species only occasionally meeting, but then producing polymorphic progeny. They consider that changes of climate, as during the retreat of the ice in Europe, may cause such meetings on a large scale, and that if a segregate form happens to be later isolated the progeny will tend to become more and more homogenous. They see support for this argument in the great number of species found on the small isolated area of the Cape Peninsula, almost as many as are found in the whole of Natal. Cockayne and Allan (26) list with notes 440 groups of wild hybrids in the New Zealand flora, of which the majority are considered to be in little doubt. These, exclusive of bigeneric hybrids, are distributed among 43 families and 73 genera, involving 416 species, or about 20 per cent. of the flora. Examples of the crossing of very distinct life-forms are noted, and 12 large genera are stated to be especially rich in hybrid forms. They use the terms "swarm" for an individual polymorphic hybrid population, and "group" for the whole assemblage of forms found between two parents. They conclude from their general survey (see also Cockayne and Allan (25)) that highly polymorphic groups are not uncommon, and that fertile hybrids are common in certain genera, *Rubus* hybrids and those involving the "whipcord" group of *Hebe* (species with greatly reduced scale-like leaves) being notable exceptions. In some swarms of *Hebe* and *Leptospermum* it is impossible to sort out, by field-observations, true-breeding units.

BIGENERIC HYBRIDS

Burtt and Hill (13) describe four crosses between *Gaultheria* and *Pernettya*. Fruit characters are noted for several specimens, and one of the hybrids, at least, appears to be fertile, being abundant in certain localities. Moss (76) discusses wild hybrids in *Clematis*, *Anemone* and *Gerbera* found in the Transvaal, and concludes that both bigeneric and interspecific hybrids may be fertile. It is extremely unusual to meet with natural hybrids a con-

siderable distance away from the parents, and he finds no evidence of the origin of species by hybridization, though he considers field evidence is often decisive as to the occurrence of wild hybrids. Warren (103) gives details of a single plant found in a wild population of *Arcotis stachadifolia* and *Venidium wyleyi* that was clearly a bigeneric hybrid. He takes this as a confirmation of the view that these genera are very closely related. F. Nilsson (81) successfully crossed *Lolium multiflorum* and *Festuca gigantea*, the sole plant secured having only nine per cent. normal pollen. The vegetative characters clearly show its hybrid origin, but while *L. perenne* \times *gigantea* has been recorded as a wild hybrid on several occasions, the absence of any such record for the present cross is considered to be because the species meet rather seldom in nature. Hybrid plants of this origin arose, however, spontaneously in Nilsson's cultures. Nilsson (83) describes a natural hybrid between *Festuca rubra* and *Lolium perenne*. It is apparently almost completely sterile, but two plants were raised from seed, probably back-crosses with *perenne*. This view is supported by cytological analysis, which is further held to give evidence of the close relationship of the two species. It is notable that of the ten natural bigeneric crosses in grasses listed by Ullmann (102) seven are between *Lolium* and *Festuca*, supporting recent views that *Lolium* should be transferred from the *Hordeae* to the *Festuceae*. Holmberg (51) demonstrates that crosses between *Festuca pratensis* and *Lolium multiflorum* are not altogether infrequent, but that the pollen is mostly imperfect. Laumont (59) showed that a natural hybrid found by Perrot, between *Aegilops ovata* and durum wheat, was fertile. Some second generation plants were also fertile, and produced certain constant forms in the third generation. Murbeck (79) describes six intergeneric hybrids between *Celsia* and *Verbascum*, some being wild plants, and emphasizes the close relationship between these genera. Bad pollen is accepted as a good field sign of hybridism, but poor seed-setting may be found in pure species. Cockayne and Allan (26) list, some doubtfully, bigeneric hybrids between *Nothopanax* and *Pseudopanax*, *Ewartia* and *Helichrysum*, *Gnaphalium* and *Helichrysum*, *Helichrysum* and *Raoulia*, *Leucogenes* and *Raoulia* (four). For the most part these appear to be sterile, though the genera concerned are very closely related indeed.

INFECTION OF POPULATION

Marsden-Jones and Turrill (69) state that crossing in *Silene* has little or no effect on general populations, but in *Centaurea* hybridism affects whole populations to the extent that many so-called species are of hybrid origin, as is proved by synthetic and analytic experiments, and by extensive field-work. Floderus (44) finds that certain species of *Salix*, clear-cut in their distinctive characters in Siberia, appear but seldom in Fennoscandia and rarely in pure form. On the other hand, their hybrids with western species are more common, often occurring in masses over extensive areas, a good example being *S. herbacea* \times *rotundifolia*. Du Rietz (38) states that in Scandinavia *S. herbacea* and *lapporum* produce fertile hybrids of remarkable polymorphy. Hybrids may be found wherever the species meet, and in places may be abundant. But in general the populations of the two species are isolated, the one being a high-alpine the other a low-alpine species. There are thus "immense pure populations" with little or no chance of infection. Cockayne (24) gives an analogous case. In a few localities the forest-tree *Plagianthus betulinus* meets the salt-marsh shrub *P. divaricatus*, and produces as a hybrid small twiggy trees that may enter the forest community, "and so by degrees a new life-form and a new group of the flora have come into the forest of alluvial valleys watered by a tidal river." Du Rietz (38) refers to the geographical distribution of various characters: "in the genus *Celmisia* brown and yellow hairs showed a remarkable frequency in the northern part of South Island, being found there in species widely differing in other respects . . . but of much rarer occurrence in other parts of New Zealand." Similar groupings were noted in *Dracophyllum* (inflorescences), and in *Euphrasia* (leaf-characters). He could find no "other possible explanation than that in the syngameon originally formed by each of these genera, certain genes had been generally distributed only in certain districts, but in those districts had 'infected' the whole population." Allan (5) has drawn attention to the passing of the undulate leaf-form of a species of *Coriaria* into hybrid populations. It is now known that this "undulata" form occurs in three distinct species, two in North Island and one in South Island, and the explanation of Du Rietz for coloured hairs in *Celmisia* does not appear to meet this case very well. Sometimes "infection" is so great and involves so many

characters that the taxonomist is at the moment altogether at a loss. Du Rietz (38) instances *Betula* and *Salix* for the Northern Hemisphere, and *Alseuosmia* for New Zealand.

Wisniewski (105) made biometric studies of *Fagus silvatica* in Poland and concluded that "Die Variabilität der Buche in Polen bleibt ohne Zweifel in engerer Verbindung mit der Höhe der Standorte ü. d. Meeresspiegel." Czeczottowa (35), after a thorough-going study of the leaves and noting the fruits of *Fagus orientalis*, *F. silvatica* and their intermediates, draws the conclusion that "South-eastern and a part of Central Europe is inhabited by a beech population which in the qualitative characters of its fruits does not seem to be essentially different from *F. silvatica*, while in leaves it resembles partly *F. orientalis*, partly *F. silvatica*, and displays also its own peculiar features." This population is given the name *F. moesiaca*. Hybrids, probably infrequent, are met with where *moesiaca* meets *orientalis*. It is suggested that *moesiaca* may have resulted from hybridization during an overlapping of the areas of *silvatica* and *orientalis*, "possibly due to an impulse received during the retreat southwards of the beech in the Glacial Period." But, although possible hybrids have been met with in Transcaucasia, where the oriental and the Persian beech overlap, there is small diversity in some parts of the area of *F. moesiaca*, and "nobody has yet found a beech bearing foliaceous appendages on the cupulae (peculiar to *F. orientalis*) in the northwestern part of the Balkan Peninsula." The author favours the theory that the three species evolved from a common stock "independently from each other, on the whole space of their areas." Cockayne (24), in a discussion of hybridism in the forests of New Zealand, points out that in *Nothofagus* forests "large areas of forest are dominated by one or other of the species, while the non-crossing *N. Menziesii* and *N. fusca* frequently occur together, and it is usually only when the latter and one or more of the remaining species occur in proximity that hybrids occur. Generally in *Nothofagus cliffortioides-fusca* forests, hybrids form a very small percentage of the trees, but perhaps 5-10 per cent. occur with fair frequency. In some places, however, there may be at least 50 per cent. of hybrids, but such spots are small in extent. Nevertheless, it is clear that the hybrids can well hold their own, a statement substantiated by the presence of hybrid seedlings in great numbers beneath hybrid trees. After

a forest fire hybrids may occur in great profusion and the reinstated forest will certainly contain more hybrid trees than did the former community. Where there are small colonies of *N. fusca*, in what is otherwise a pure forest of *N. cliffortioides*, hybrid trees occur and such, of seed-bearing age, I have seen on a stony river-bed, 96 km. or more below the last tree of *N. fusca*, so establishing a nucleus round which hybrids will spread into new territory and dominate." More general statements of the ecological importance of wild hybrids will be found in Cockayne and Allan (25) and Allan (5). Of value, too, is the paper by Marsden-Jones, Summerhayes, and Turrill (72) on special herbaria for genetic and other special purposes.

LARGE-SCALE POLLEN STUDY

Rather apart from other papers is that of Lawson (60). Accepting abnormality in pollen as a criterion of hybridity, he examined the pollen in twenty-three species (12 genera) of Proteaceae, and in twenty-eight species (8 genera) of Myrtaceae. Averaging his figures we find that in the Proteaceae there is 60 per cent. of bad pollen, ranging in the different species from 95 to 25, and in the Myrtaceae 61, from 95 to 30. He is thus led to conclude that "as the types whose pollen has been described in the present paper cover such a wide range of genera and species of both those large families, which represent the predominant portion of the endemic flora of Australia, it seems a reasonable hypothesis that hybridization has been of no uncommon occurrence in the evolution of these two great tribes. I am quite convinced that hybridization occurs wherever possible, and the possibilities, in these two tribes at least, are general." The conclusion appears too massive for its slender base, and further studies on this aspect are desirable.

CONCLUSION

It is beyond the scope of this article to discuss cytological and genetic work based on wild plants in general, though certain aspects have been referred to. Obviously, any complete view must take such work into consideration. Probably the chief value of field study at the moment is to suggest individual plants and groups that are most likely to repay cytological analysis and genetic study. The articles by Lindstrom (64) and Sax (96) should be studied in this connection. Not cited in these is the remarkable study by Gershoy

(47) and his co-workers on the North American violets, extending the classical studies of Brainerd. Deserving of note, too, is the paper of Auseklis and Zamelis (9) dealing with *Viola* problems, and especially with the artificially produced constant hybrid *V. arteficiosa*, derived from the crossing of *V. bosniaca* and *V. arvensis*, which shows very marked patrocliny. Nor is there space to deal with spontaneous hybrids, arising accidentally or from deliberate planning, in cultivated crops, and what Turesson (101) calls "semi-natural" populations. That such work may yield data of importance is shown by the papers of Marsden-Jones and Turrill (70) on a tetraploid saxifrage, of Okonogi (91) on a *Brassica* cross, Henry and Chih (49) on flax hybrids, and of Williams (104) on red clover. Good surveys are provided by the books of Darlington ("Chromosomes and Plant-Breeding") and Crane and Lawrence ("The Genetics of Garden Plants").

Lotsy (67) raised the questions: "(1) Is hybridization of sufficiently frequent occurrence in nature to account for an appreciable part of the diversity everywhere observable? (2) If so, how large is that part?" The study of wild hybrids in the field, though it has settled no major problems, has already gone a long way towards suggesting that the answer to Lotsy's first question is "Yes," and that a tentative answer to the second is "at least not insignificant." It has been established that hybrids occur in a great range of genera and families, have all grades of fertility, may exhibit characters not revealed in the parents, may produce polymorphic progeny for several generations, may produce (perhaps rarely) constant new forms, and may play an important part in plant communities. Most botanists will agree that it has been shown that the phenomena of wild hybridism cannot be neglected as of little moment by the taxonomist, the ecologist or the geneticist. We are not yet provided with a sufficient body of knowledge to do much more than speculate on its bearing on the larger questions of geographical distribution, family and generic differentiation, and evolution in general. Much more work is required before we can definitely evaluate the suggestive hypothesis of Turesson (100, and later papers) that "ecotypes" are selected out by climate and habitat from previously undifferentiated heteromorphic populations. Whatever modifications may have to be applied to the hypothesis, the wealth of data accumulated by Turesson on the habitat distribu-

tion of different forms of a great many species remains impressive. *Cotyledon* in South Africa and *Hebe* in New Zealand suggest themselves as fields for study. It is already known that certain species of *Hebe* show varieties with fairly clearly defined geographical bounds. How far the classification, based largely on his important studies on *Rumex* hybrids, of Danser's (36) "Bastardierungs-genossenschaft" into *comparia*, *commiscua* and *convivia*, according to their powers of inter-fertilization, is likely to prove fruitful remains for future work to decide. Observations on wild and artificial hybrids both show that many quite unexpected pairs do cross or can be crossed. On one point, however, Müntzing (78) represents the views of many when he states, "It is now perfectly clear that allopolyploidy has played an important rôle in the evolution of plant species at least amongst the angiosperms."

It is only by careful examination of the whole body of evidence that the student of any branch or "track" can safely detect the real lines of the advance to biology as an exact science that Heribert Nilsson (88) says we are making. And after the excitement of discovery we will do well to ponder that hard saying of his: "May it not be so that evolution is a stately edifice that we ourselves have erected, laid stone on stone and is now completed—and like every other building, dead?"

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GLOSSARY

- allopolyploid: a polyploid having unidentical sets of chromosomes.
- amphidiploid: a hybrid possessing $2n$ number of chromosomes from one parent and $2n$ from the other parent.
- amphipolyploid: includes amphidiploids and hybrids with higher n numbers from the parents.
- amphipolyploid: includes amphidiploids and other hybrids with higher n numbers from the parents.
- autopolyploid: a polyploid having identical sets of chromosomes.
- cleistogamy: the condition of close fertilization taking place within unopened flowers.
- heterozygosity: the state of possessing contrasting factors for any number of characters.
- jordanon: a species in the narrow concept of Jordan based on minute differences.
- linneon: a species in the broad concept of Linnaeus including minor variations.
- polyploid: an organism with more than two sets of homologous chromosomes.

THE RÔLE OF VITAMINS IN PLANT DEVELOPMENT

JAMES BONNER

William G. Kerckhoff Laboratories, California Institute of Technology

INTRODUCTION

When one hears the word "vitamin" one ordinarily thinks of accessory dietary factors which are necessary for animals. One thinks, in general, of plants in this connection only as sources of vitamins. We know that we should eat green leaves, unpolished rice, whole wheat, and citrus fruits to obtain, among other things, our vitamins. It would seem unlikely, however, that a seed should contain, say, vitamin B, merely that animals might, at the seed's expense, prevent an avitaminosis. We might well enquire whether these accessory substances do not play a rôle in the economy of the plant.

In the past few years a small amount of quite definite evidence has been obtained which shows that certain of the vitamins really do play a rôle in plant development. At the same time it has been found that accessory substances not as yet known to be of significance for the animal are closely associated with the vitamins in their plant activities. It is necessary, therefore, to include in this discussion the group of the "bios" substances, and also to include them temporarily under the head of vitamin. This is perhaps permissible inasmuch as the bioses bear much the same relation to plants as do the vitamins, or as do the vitamins to animals. The more general name of "accessory nutritional substances," or, simply, "accessory substances" might also be applied to these special factors which are needed by the plant in small amounts. The collective term "nutrilites" has been used in recent years by Williams (146) with particular reference to the accessory substances necessary for plant growth.

Auxin, commonly known as "the plant growth hormone," will be omitted here since a discussion of its physiology has already appeared in this journal (135). Auxin is of course a growth factor inasmuch as it is essential, in minute amounts, for increase in volume of the plant cell. It is, however, also a typical hormone since it is a carrier of correlation, a "correlation substance."

A brief discussion of still another class of substances, namely,

the oestrogenic sterols, will also be included here. These substances, although they are animal hormones, act, nevertheless, as vitamins in plant development.

Plants may be either heterotrophic or autotrophic with respect to accessory substances. There are, for example, species of molds and of bacteria which are heterotrophic with respect to one or more special growth factors. For these species, as for animals, the accessory substances must be supplied from the outside, either completely or essentially preformed. There are, on the other hand, a great many completely autotrophic species. These species do not require for their normal growth the *addition* of vitamins to their culture media, and they do not respond to such additions. This does not mean that the organism in question is able to live without the particular growth factor. It means, rather, as has been quite definitely shown, that the autotrophic organism is autotrophic because it is able to synthesize for itself the accessory substances.

BIOS

The accessory factor relationships of a few microorganisms will be taken up first, in particular, the case of yeasts which is historically the oldest and at present the best understood. In 1860 Pasteur in his "Mémoire sur la Fermentation Alcoolique" (76) showed that yeast might be grown upon a synthetic medium consisting of only ammonium salt, sugar, and ash of yeast. A few years later Liebig (57) made, however, strenuous objections. Even with the strictest observance of Pasteur's conditions the yeast of Liebig would not grow in a normal fashion. This was quite a paradox at the time; the experiment was simple and each of the experimenters reliable, yet they obtained diametrically opposed results. Today, however, it seems very probable that they were in fact both right. Liebig died before the question could be settled and the authority of Pasteur carried his pronouncement down to the year 1901. In that year Wildiers (140) showed that, if the inoculation is sufficiently small, yeast will not grow normally upon a completely synthetic medium. There must be added a small amount of a heat-stable, organic substance which may be extracted from yeast. He called the "life-giving" substance "bios" but expressed the hope that a chemical name might eventually replace this. Wildiers also proposed one possible explanation of the difference between the

findings of Pasteur and of Liebig. Pasteur may have used a relatively large inoculation so that the inoculum carried over into the fresh medium enough bios to start the new culture. The unfortunate Wildiers was far ahead of his time, since the mechanists of that day found the concept of "bios" to be rather too vitalistic. It was said, for example, that Wildiers' results might better be explained as being due to traces of heavy metal impurities in his medium, these impurities being bound as complexes by the protein of the yeast extract.

A period of relative quiescence in the bios field reigned until the 1920's. At that time, however, intensive work, marked by contradictory results and opinions, was resumed. After the discovery of vitamin B it was soon found that yeast contains considerable amounts of this substance, and in fact is able to synthesize it (30). It was assumed by Williams (142, 143) and others that vitamin B and bios are identical—an assumption which in its original form was soon shown to be incorrect (20, 26, 27, 69). (Vitamin B₁ is, however, *one* of the bios factors, as will be discussed below.) Fulmer, Nelson and White (28) and the school of Miller (62, 63) first really opened the way for the clearing up of the bios problem. The former showed that one particular variety of yeast with which they worked was capable of indefinite, nearly normal, growth on a purely synthetic medium. Lucas (60), a student of Miller, showed, however, that different races of yeast vary in their response to added bios. A yeast which grew relatively well on synthetic medium gave little response to added bios, whereas a yeast which grew very poorly on synthetic medium gave a large response to such additions. More recently, the differences between yeast strains and species have been studied in more detail by Williams, Warner and Roehm (145), Coping (14), Stantial (126), Williams and Saunders (151) and Farrell (24). In general, it has been shown that there are both qualitative and quantitative differences in bios requirements. Wild yeasts which grow well and ferment badly do not only not need added bios, but actually produce it themselves in large amounts. Highly cultivated yeasts, on the other hand, require for good growth of a culture, the addition of bios to the medium.

The multiple nature of bios was demonstrated by Lucas (60) who showed that the growth-stimulating extract contains at least two fractions, bios I which gives an alcohol-insoluble barium salt,

and bios II which does not give such a salt. Each fraction had but little activity alone; if the fractions were recombined, however, the original activity was restored. The schools of Williams (144), of Miller (65) and of Kögl (44) have now found that bios II itself is multiple in nature. The bios II fraction may be separated with Fuller's earth into an adsorbed fraction, replaceable by vitamin B₁, and an unadsorbed fraction, the "pantothenic acid" which has been greatly purified by Williams and his co-workers (148, 150). Or, bios II may be separated by charcoal. The adsorbed fraction is the "biotin" of Kögl and Tönnis (see below) and the unadsorbed fraction, the bios III of Kögl and Tönnis (the bios IIa of Miller *et al.*, 65) is in this case again possibly vitamin B₁ or a closely related substance.

As regards the chemistry of the bios complex, Eastcott (18) found bios I to be i-inositol, and this has been confirmed by many workers. Of particular importance, however, is the recent isolation of bios II in pure form by Kögl and Tönnis (44). In an excellent piece of work, Kögl and Tönnis have not only studied the physiology of bios in some detail, but have also succeeded in isolating in crystalline form the fraction of bios II which, as mentioned above, is adsorbed on charcoal. To this they have given the name of "biotin." It is not necessary to go into the details of the testing methods and of the isolation procedure which they used; it must suffice to point out that they commenced with 250 kgs. of dried yolk of egg (a rich source of bios II) and ended with 1.1 mgs. of recrystallized biotin, an amphoteric substance of molecular weight about 200. Biotin is one of the most potent of the known accessory substances, a solution of one part in 4×10^{11} still possessing marked activity on yeast.

The relationship of biotin to the pantothenic acid of Williams is as yet somewhat uncertain. The two are very similar in many respects, although in certain physiological tests they do possess somewhat different activities. A final conclusion as to their identity or non-identity can come only from better knowledge of the chemical structure of each. There is little doubt, however, that the bios IIb of Miller is identical with biotin (44, 64).

There are, then, three separable and isolatable constituents of Wildiers' "bios," namely, bios I or i-inositol; bios II, biotin or pantothenic acid; and bios III, which is most probably vitamin B₁.

That there may be still other bios fractions for some yeasts or for special cultural conditions of one strain, has been indicated by Farell (24) whose bios V is apparently vitamin C, and by Williams and Rohrman (153) who have found that alanine in considerable dilution (one in 10⁷) markedly increases some yeast crops.

It was mentioned earlier that while wild yeasts form their own bios, highly cultivated yeasts demand an outside supply. This statement now needs some qualification. There are strains of yeast which form for themselves some bios II and some bios III and which lack principally bios I (inositol). There are also strains of yeast which respond to vitamin B₁ (bios III) alone, and which hence form at least some bios I and some bios II. The most strikingly vitamin heterotrophic strains, however, are those which lack bios II, and which respond to its addition to the culture medium by a great increase in growth. Wildiers' original yeast was of this kind. The addition of bios I together with bios II, or the combination of all three, may still further increase the effect. This interaction of factors in strains heterotrophic for different members of the bios group may be seen particularly clearly in the work of Williams and Saunders (151). In general, a yeast may be completely vitamin autotrophic, or it may be heterotrophic for any one or more accessory substances. If a yeast is heterotrophic for two accessory substances, as, for example, biotin and inositol, the influence of one may be evident only in the presence of the other. In the race M of Kögl and Tönnis, inositol is inactive except in the presence of biotin. This principle of the interaction of a number of accessory substances is of great importance, and will be of still greater importance in the study of the accessory factor relationships of other organisms. We owe a great deal to the work of Miller and his school (62, 64, 65, 126, 24) and to Williams and Saunders (151) that it has been even partially worked out for the different varieties of yeasts.

ACCESSORY GROWTH SUBSTANCES FOR FUNGI

It was pointed out by Linossier (58) in 1919 that many molds also require vitamin-like substances for their growth in culture. The general rule appeared to be that a species which required such accessory substances to start the growth of the culture, produced such substances itself once the culture became well established. Only a few molds were never able to produce their own accessory

substances. Willaman (141) also found that *Sclerotinia cinerea*, the brown rot of fruits, is unable to grow on pure synthetic medium but demands vitamin-like substances from fruit juice. Other investigations, such as those of Lepeschkin (56) and of Williams and Honn (149), have also indicated that many fungi are not vitamin autotrophic, but elucidation of the question is due primarily to the work of Schopfer in the past six, and particularly in the past three years.

Schopfer (97 to 123) found that *Phycomyces Blakesleeanus* can not grow on a pure synthetic medium, but that organic material, such as yeast, wheat or green leaf extract, must be added. The active substance resembled vitamin B_1 in its chemical properties. Schopfer (99, 105, 108) and independently Burgeff (11) therefore tried crystalline B_1 and found it to be highly active, as little as five in 10^{10} sufficing to bring about some growth. Vitamin B_2 (lactoflavin), although chemically a completely different substance, is able to replace B_1 to some extent, although it is less active (99) and its activity may be due to B_1 as an impurity. Schopfer and Burgeff have gone on to show that fungi, like the races of yeast, may be divided into the autotrophs and the heterotrophs from the standpoint of vitamin B_1 . A few examples of species in the two groups are given in Table 1. The autotrophs synthesize B_1 (or a substance having the same physiological activity) from inorganic materials and sugar. The heterotrophs must have vitamin B_1 pre-formed.

There are a number of other accessory substances for the growth of fungi, although none of them has been so well studied as has vitamin B_1 . Büning (9, 10) has shown that although B_1 is without effect upon the growth of *Aspergillus niger*, B_2 possesses a marked stimulating effect. Schopfer (107, 119) and Schopfer and Moser (123) have also demonstrated that there is a "factor M" (possibly of multiple nature) in wheat germ, and that this factor, which is not B_1 , increases the growth of the B_1 autotrophic *Rhizopus*. Nielsen (71) and Nielsen and Hartelius (72, 73) have found that *Rhizopus* cultures produce a substance beneficial for the growth of *Aspergillus*. This substance they call "growth substance B." It is apparently of a relatively simple nature since it can be formed by merely autoclaving together tartaric acid and filter paper, the filter paper supplying an inorganic co-factor. The

chemical nature and the place in the accessory substance scheme of this "growth substance B" is, however, at present unknown.

The complicated case of a fungus heterotrophic with respect to several substances has been studied by Mosher, Saunders, Kingery and Williams (68) with the parasite *Trichophyton interdigitale*. This mold requires very special nutrition, the reason, of course, for the fact that it normally lives under quite special conditions. A satisfactory medium for its cultivation must contain:

1. sugar: any common one except lactose.
2. inorganic ions: K, NH₄, Zn, Mn, Mg, Fe, Cu, Ca, PO₄, SO₄, Cl.
3. amino acids, which are essential or nearly so: leucine, aspartic acid, a-amino-b-hydroxy-n-butyric acid, proline, valine, lysine, phenyl alanine.
4. accessory substances.

Of the last, four are recognized, vitamin B₁, vitamin B₂, bios I (inositol), and pantothenic acid (bios II). Any one of these substances if added to the medium in the correct concentration will enable the mold to start growing slowly. After it is well established it then produces the others for itself. Two of the accessory substances are better than one, three are better than two, and with all four the fungus grows best of all.

ACCESSORY GROWTH SUBSTANCES FOR BACTERIA

The present status of our knowledge concerning the accessory growth factors of bacteria will be considered very briefly. It has long been known that many bacteria require, for their cultivation *in vitro*, extracts of one kind or another. Just as was the case with the earlier work on fungi, much of the work upon the nutritional requirements of bacteria, particularly of the pathogens, has been done with a view toward finding suitable culture media rather than with a view toward the elucidation of the nature of the various accessory substances involved. The nature of the latter has hence remained obscure. A recent and comprehensive review of the rather chaotic earlier literature may be found in Peskett (77). In general, it has appeared that water-soluble growth factors resembling but not identical with vitamin B are among the active principles (81, 17, 77). In *Streptothrix*, which has been studied more extensively by Reader (81, 82), mannitol plays the part assumed by

inositol in yeast, that is, the part of bios I, inactive or little active alone, but capable of increasing the activity of a bios II. The bios II is in this case a "Streptothrix factor," closely associated with, perhaps identical with, vitamin B₁ (78).

Tatum, Wood and Peterson (127) have studied the needs of propionic acid bacteria in culture. Beside the usual nutrients this organism must have two special factors, one ether-soluble and not as yet further identified. The other is ether-insoluble and is identical with vitamin B₁. The vitamin is highly active, as little as 5 in 10⁹ being sufficient to elicit growth response.

Knight and Fildes (43) have shown that there is an accessory substance necessary for the growth of *B. sporogenes*, the "sporogenes vitamin." This is a highly active substance and apparently quite different from any of the accessory substances heretofore discussed. Pappenheimer (79) has isolated the substance from the urine of cows and found it to be an unsaturated hydroxy acid of molecular weight approximately 200. It possesses activity at a concentration of 4 in 10⁸. Saunders and co-workers (90) also have enriched a water-soluble growth factor for pathogenic bacteria. It has not as yet been obtained in the pure state and its nature is unknown.

SYMBIOSIS AND VITAMIN INTERACTION

The fact that vitamin autotrophic microorganisms produce accessory growth factors may well clarify very considerably our conceptions regarding the physiology of symbiosis and the nature of the mutual interaction of symbionts. This is well exemplified in the work of Burgeff (11). As is well known, the orchids of the *Cattleya* group can be germinated and grown in the seedling stage in the absence of the mold symbiont (*Rhizoctonia repens*). Orchids of the more highly specialized *Vanda* group, on the other hand, develop very poorly in the absence of their symbiont (*Rhizoctonia mucoroides*), although in its presence they germinate and grow very well. Burgeff found that dead symbiont is quite as effective as living, and has in fact shown that the active principle, normally formed for the seedling by the fungus, is an accessory substance resembling bios II, although it has not as yet been chemically identified. The orchids of the *Vanda* group are, according to Burgeff, heterotrophic with respect to this substance, at least in the seedling stage. This growth factor-lack is normally

made good by the autotrophic fungus. This of course by no means exhausts the activities of the symbiont, but it is apparently an important function.

Other examples of accessory substance interaction between microorganisms and higher plants have not yet been studied in great detail. McBurney, Bollen and Williams (61) have found, however, that the nodule bacterium, *Rhizobium*, produces bios II, and that a part of the beneficial effects of nodule bacteria upon legumes is indeed to be ascribed to the bios taken up by the host plant. Thimann (129) considers it probable that the formation by the host plant of the nodule itself is due to the local production of auxin (135) by the invading *Rhizobium*. Callus-forming substances such as are sometimes produced by bacteria may also be of an auxin nature. Reference might also be made to Mockeridge (66) who has demonstrated that bacterial substances of a bios-like nature may increase the growth of *Lemna*. It may well be, as will be shown below, that soil microorganisms affect the growth of the roots of higher plants because of the production into the soil of vitamin B₁ by the former, the vitamin B then acting as an accessory growth factor for the root.

ACCESSORY GROWTH SUBSTANCES FOR HIGHER PLANTS

We will now turn to the vitamin relations of the higher plants. Bottomley (6, 7) first indicated the importance of accessory substances for the growth of higher plants. He found, for example, that peat, when acted upon by bacteria as well as the extract of wheat seedlings, possesses a considerable beneficial effect upon the growth of *Primula*, of wheat seedlings and of *Lemna*. These "auximones" of Bottomley were probably of diverse nature. In addition to bios, vitamin B, etc., his extracts contained of course inorganic material which may have been the beneficial principle in some cases. One must also remember that green plants, growing in the light, may be expected to be largely vitamin autotrophic, since they synthesize vitamins to meet not only their own requirements but also to meet those of the vitamin heterotrophic organisms. In order to demonstrate clearly that vitamins actually do play a rôle in the economy of higher plants it may often be necessary to make use of abnormal conditions under which this vitamin synthesis does not take place. Thus, plants might be placed in darkness or at-

tempts might be made to isolate heterotrophic organs from the autotrophic leaves. The application of such methods will be discussed in more detail below.

In the work on higher plants, as in that upon microorganisms, interest has centered upon the water-soluble rather than upon the fat-soluble vitamins, so that very little of the rôle of the latter is known at present. Provitamin A, or carotin, plays of course some part in the general economy of the plant, and a relationship to photosynthesis has been frequently suggested (67). Of more interest from a developmental standpoint is the observation of Lazar (54, 55) that carotin may act as a "root-forming substance." Lazar found that the regeneration of roots upon cuttings of *Impatiens* hypocotyls may be greatly speeded up and the final number of roots increased if the hypocotyls are first placed for a short time in medium containing carotin. The rôle of vitamin E in the plant also remains obscure, although this substance is widely distributed in plant tissues (22) and is readily synthesized by the growing plant (23).

Vitamin C is found generally in plant tissues. External or nutritional conditions, such as the quality or amount of available nitrogen or phosphorus, which affect the growth of the plant also affect the synthesis of vitamin C by the plant. If growth is slower, synthesis of vitamin C is slower, and, as has been shown by Virtanen, von Hausen and Saastamoinen (130), optimal growth and maximal C content coincide under most conditions. In the dry or dormant seed vitamin C is not present, but large amounts are formed upon germination, as shown first by Fürst (29) and later by Harden and Zilva (31), and by Kucera (49, 50) among others. In fact, increase of vitamin C content is in some seeds, such as barley, one of the earliest indications of germination. As germination progresses the C content of the seedling rises to a maximum where it remains for some time. This may be clearly seen from the work of Ray (80) with the pea seedling. The vitamin is synthesized, even in the dark, from sugars mobilized from the cotyledons, a question which has been more thoroughly investigated by Ray (80). Ray cultured pea embryos *in vitro* upon media containing different kinds of sugar as the carbohydrate source. Although vitamin C could be synthesized, under these conditions, from a variety of hexoses and hexose-containing disaccharides, man-

nose seemed to be the most favorable. In the work of Ray, again, good growth was correlated with large synthesis of vitamin C. The reverse did not always hold true, as would be expected, since, as will be apparent below, vitamin C is only one of a number of factors which are necessary for the growth of the pea seedling, or of the pea embryo *in vitro*.

The correlation of good growth with high vitamin C content is perhaps only a meager indication of a causal connection between the two. It has, however, sufficed to induce more extensive investigations. Havas (37) was able to increase the growth rate of wheat seedlings by the addition of C, and he also indicated that plants may differ in their response, oats and tomato being less responsive than wheat. This may be interpreted as an indication that the oat and the tomato are more vitamin C autotrophic than is the wheat seedling. Havas also suggests that vitamin C may play a rôle in the development of abnormal plant growths (38).

Von Hausen (36) reasoned that if vitamin C does actually play a part in seedling growth, one might be able to speed up germination by increasing the C content of the seed. This he did by soaking pea seeds in a concentrated solution, from which he showed that the vitamin was actually absorbed. He then grew seedlings from such treated seeds. In the initial stages the treated seed increased in dry weight 35% faster than the controls. If more C was added to the culture vessels at intervals of ten days, increases of as much as 100% were obtained. When young plants were deprived of their cotyledons and placed on sterile agar the effect was even more striking, the treated plants increasing in length up to two and one-half times as fast as the controls. In similar experiments carried out in this laboratory smaller effects of vitamin C were obtained. Pea seedlings grown in the dark in small bottles were deprived of their cotyledons, decapitated, and sugar solutions added to the roots. Since the plants are decapitated the axillary buds grow out. If vitamin C is also added to the solution surrounding the roots, the axillary buds do grow out somewhat faster than do the controls. At the same time, their growth rate is still far below that of the plants whose cotyledons remain.

Bonner and Axtman (5) have shown that vitamin C, when added in the correct concentration to the medium, definitely increases the growth rate of excised pea embryos of the variety "Perfection."

Kögl and Haagen-Smit, on the other hand (45), have reported that there is no influence of vitamin C on the growth of excised pea embryos of the varieties "Kaapsche Groene" and "Kortstroo Schokkers." This difference in behavior should be investigated further, but it may well indicate that the varieties of peas differ in their ability to synthesize vitamin C for themselves, that is, that some varieties are more C autotrophic than others. The C heterotrophic races would obviously be the more suitable for further study of the rôle of vitamin C in plant development.

Biotin, or more generally, bios II is, it seems probable, a growth factor of the first importance for higher plants. It occurs practically universally in plant tissues (also in animal tissues), having been found in leaves, stems, roots, seeds, etc., of a large number of species. Its occurrence shows a striking parallel to that of vitamin B₁. Thus, in rice biotin is found principally in the aleurone layer and is removed, as is B₁, during polishing of the rice (45). In the pea the bulk of the biotin is found in the cotyledons, although its actual concentration is higher in the embryo. Upon germination bios II is mobilized from the aleurone, endosperm or cotyledon, and passes into the growing seedling. Similarly, during the sprouting of potatoes, bios II is mobilized by the young shoot. From these facts one might infer that bios II is of significance in seedling growth. That this is the case has been directly demonstrated by Kögl and Haagen-Smit (45). These workers used, again, excised pea embryos cultivated upon a nutrient gelatine medium. The addition of .08 gamma of biotin per embryo resulted in an increase in shoot dry weight of 63% over that of the control. The effect was specifically upon the shoot, the root being completely unaffected. The biotin reserve of one pea seed was found to be approximately .1 gamma. We have here, then, an effect of pure biotin in physiological amounts. An effect of pantothenic acid, similar to that of biotin, upon the pea embryo, has been reported by Bonner and Axtman (5).

Williams and Rohrman (152) have shown that pantothenic acid, the bios II of Williams, may cause similar responses in other plants. They worked with the liverwort, *Ricciocarpus*, which, although a green plant and presumably vitamin autotrophic, responds, nevertheless, to the addition of pantothenic acid with a marked increase in growth. For this effect, pantothenic acid in the concentration

of one in 10^8 was necessary, a concentration of the same order as that used by Kögl and Haagen-Smit. A similar improvement in the growth of sterile alfalfa plants upon the addition of pantothenic acid has been noted by McBurney, Bollen and Williams (61). These, while isolated observations, are of considerable interest since they suggest that the growth of a green plant may, nevertheless, be limited by accessory growth factors which may be supplied from the outside.

Dagys (15, 16) has recently studied the distribution of bios II in plant tissues and has found a number of interesting correlations with growth activity. At the time that tree buds emerge from their winter dormancy there is a large increase in their content of bios II. This high level is then maintained during the summer, to sink again to a minimum at the time of deepest dormancy. Young actively growing leaves contain more bios II than do mature leaves. Dagys also has confirmed the mobilization of bios by the germinating seed.

✓ Vitamin B₁, also, is of general occurrence in the tissues of higher plants. It has been found in leaves, stems, roots, fruits, seeds, etc. (Chick and Hume, 13; Bucek, 8; Kucera, 49, 50, 51; Hlavaty, 39; Baker and Wright, 2; summary in Sherman and Smith, 125). In the seed it may either be stored in the aleurone as in barley, wheat and rice, (in which case it is removed during "polishing," Eijkmann's original discovery, 19), or it may be present in the cotyledons as in the pea. During germination the vitamin disappears from the aleurone or cotyledons and is presumably used by the seedling (Kucera, 49, 50, 51; Hlavaty, 39). Before the vitamin supply of the seed is completely exhausted, fresh production by the leaves commences if the plant is in the light (Hlavaty, 39).

The first direct demonstration that vitamin B₁ is a growth factor for higher plants was that of Kögl and Haagen-Smit (45) although the work of Bonner (3) and of Robbins and Bartley (88) was nearly simultaneous with and independent of theirs. Kögl and Haagen-Smit used, again, excised pea embryos grown *in vitro* on nutrient medium. As mentioned above, they found that biotin (bios II) considerably improves the growth of such embryos and that this influence is specifically upon the shoot, since both length and weight of the root remain unaffected. This is in agreement with the observation of Robbins and White (87) that pantothenic

acid (also bios II) has no effect upon the growth of excised roots *in vitro*. Added vitamin B₁ also considerably improved the growth of the pea embryos of Kögl and Haagen-Smit, even in concentrations as low as one in 10⁸. The effect in this case was primarily upon the root, the length, weight, and branching of which was greatly increased. At the same time the length of the shoot was increased, this being possibly, however, only an indirect effect and by way of its influence upon the root. The beneficial action of vitamin B₁ upon the growth of pea embryos *in vitro* was also found by Bonner and Axtman (5). In their experiments 0.1 gamma per cc or one in 10⁷ proved to be the optimum concentration, this concentration giving an increase of shoot length of as much as 78%.

Biotin and B₁ together better the growth of the pea embryo more than does either factor alone (45) and the same holds true for the combination of pantothenic acid and B₁ (5). In addition to these two factors there are of course many more since even with bios II and vitamin B₁ in addition to the ordinary nutrients (sugar, inorganic salts) the growth is much slower than that of the normal pea seedling. One of these factors is vitamin C, as has been mentioned above. Oestrone is apparently another (5, 45), indicating that at last a specific function for the "phyto-sterols" has been found. Bios I or inositol is inactive alone or in the presence of adequate amounts of vitamin B₁ (5). Its behavior in the presence of adequate amounts of the other known accessory substances for the growth of the pea embryos has not as yet been investigated.

Since it is upon root growth that vitamin B₁ shows its most striking effects, it will be of interest to examine this more closely. It has long been known, from the work of Robbins (83, 84), of Robbins and Maneval (85), and of Kotte (48) that excised root meristems may be cultivated *in vitro* if the proper medium is used. Some growth is obtained in pure synthetic medium, but better growth may be had if yeast extract is added. White (138) was able, after he had worked out in some detail the optimal conditions for cultivation (136, 137), to grow tomato roots *in vitro* through unlimited transfers;—that is, to periodically remove small pieces of apical meristem to fresh medium, which then grew with undiminished vigor. The medium which he found to be necessary consisted of inorganic salts, sugar and yeast extract. The latter

was absolutely necessary. More recently Bonner and Addicott (4) have worked out the cultural conditions for the pea root and have been able to carry such roots through many weekly transfers without decrease of growth rate. These pea roots also require yeast extract, since without it the growth rate decreases to zero after three transfers. The factor in yeast extract which is of primary importance for the pea root was found to be vitamin B₁. Pea roots were kept growing in culture for three months (12 transfers) on a medium consisting only of organic salts, sucrose, and vitamin B₁ at a concentration of one in 10⁷. Robbins and Bartley (88) independently found that tomato roots also respond strongly to vitamin B₁ and that tomato roots may be grown *in vitro* for long periods with B₁ as the only accessory substance.

Vitamin B₁ is then, for the culture of roots, an important constituent of yeast extract, but it is not the only accessory factor which roots obtain from it. Roots grown with B₁ as the only accessory substance ultimately grow at a rate slower than that of roots grown with complete yeast extract (4). White has stressed (139) the importance of "essential" amino acids which the root may require in small amounts and which it is unable to synthesize for itself. Robbins, White, McClary, and Bartley (86) on the other hand consider that the inorganic constituents of yeast are of significance. In the case of pea roots a mixture of crystalline pure amino acids is able to replace most of the effect of yeast extract which is not due to vitamin B₁, whereas yeast ash is without effect (Bonner and Addicott, 4). It is of course possible that other accessory substances may play some rôle. Vitamin B₂ is indeed able to substitute for B₁ to some extent, as it was in *Phycomyces*, but added B₂ does not augment the effect of B₁ and the activity of B₂ may, then, be due only to the presence of B₁ as an impurity (4). As has already been mentioned, bios II has been reported to play no part in root growth. Inositol is also without effect under the conditions thus far tested.

The root is, then, an extremely heterotrophic organ. Not only does it depend upon the upper portion of the plant for its supply of carbohydrates, but also for at least a portion of its amino acids, for its accessory growth factor, vitamin B₁, and also probably for its auxin (Fiedler, 25). /

Vitamin B₂, either as flavin itself or combined with protein as the

"yellow ferment" of Warburg and Christian (132, 133), is of course an essential part of one of the plant's oxidative mechanisms. It is accordingly very widely distributed in their tissues (Kuhn, Wagner-Jauregg, and Kaltschmidt, 52; Aykroyd and Roscoe, 1; Theorell, 128, summary in Sherman and Smith, 125). Flavins identical with the animal products have been isolated from a number of plant sources such as flowers (Karrer and Schröpp, 41), seedlings (Karrer and Schröpp, 42) and green leaves (Kuhn and Kaltschmidt, 53). As is well known, vitamin B₂, flavin, has a powerful growth promoting activity on young animals and is in fact assayed in this way. At present however, no marked effects of added B₂ upon the development of higher plants have been discovered. This means only that the higher plants are then in general autotrophic for this important substance.

It is perhaps appropriate to discuss here briefly the question of the growth promoting activity of the animal sex hormones. That the female sex hormone of mammals does actually occur in plants was demonstrated by Loewe, Lange, and Spohr (59) who found that flowers possess more oestrogenically active substances than do other parts of the plant. They therefore suggested that such substances might also play the rôle of "plant sex hormones." Actual isolation of female sex hormone from plant material has been accomplished by Skarzynski (124) and by Butenandt and Jacobi (12). Since such substances do occur naturally there is, then, no reason to suppose that they do not possess some physiological rôle in the plant. Schoeller and Goebel (94, 95, 96) accordingly found that progynon (a commercial, impure preparation of follicle hormone) accelerates the flowering of *Calla* and of *Hyacinthus* if the bulbs of these plants are placed in solutions containing the hormone. Their experiments could not, however, be repeated by Virtanen, von Hausen, and Saastamoinen (31), Euler and Zondek (21), or by Harder and Störmer (32, 33). At the same time, results similar to those of Schoeller and Goebel were obtained by Janot (40) with hyacinth and by Scharrer and Schropp (91) with peas and cereals, as well as by others (134). These differences in results are apparently due to differences in the conditions under which the experiments were carried out. Janot, as well as Harder and Störmer, and Scharrer and Schropp obtained their most striking results with water cultures in which distilled water was used in

making up the medium. Those investigators who have obtained negative results have in general used tap water of varying composition. This has lead Harder and Störmer (34) to suggest that in the latter cases the hormone was converted into some inactive form. In any case, the more recent experiments make it clear that there certainly are some conditions under which follicle hormone can act as an accessory growth factor, not only for isolated embryos, (see above) but also for the normal plant. In a continuation of their experiments Schoeller and Goebel (96) found that crystalline follicle hormone added to pot cultures of *Primula*, *Fuchsia*, *Chrysanthemum*, and of various other plants, increased the number of flowers, and, in the case of tomatoes, increased the yield of fruit, above that of the untreated controls. It now seems certain however that follicle hormone does not affect flowering specifically but that the influence is indirect and by way of a general improvement in vegetative growth. Orth (75) has shown, for example, that the sexual expression of hemp, of *Mercurialis*, of corn, and of a number of lower plants is unaffected by follicle hormone, although he showed that at the same time there is an increase in vegetative growth. Harder and Störmer (33, 34, 35) also come to the conclusion that follicle hormone affects flowering in no specific fashion. In water cultures of corn vegetative growth was considerably increased by weekly additions of 300 "mouse units" of progynon. The time of flowering was not influenced. Scharrer and Schropp (92) have carried out experiments with potted plants using a series of concentrations of follicle hormone. Wheat responded to the additions vigorously with increases both of grain and of straw yield. Rye responded somewhat less, barley still less, and oats not at all. It would seem then that plants also differ in their ability to cover their own sterol requirements by synthesis.

That the application of follicle hormone to crop plants may be of practical importance is suggested by an experiment of Scharrer and Schropp (92) in which beans responded to a single addition of 1000 mouse units with an increase in yield of 39%. It is also of interest that the growth stimulation in the early stages and the increase in final yield do not parallel one another, the former being in general more striking than the latter. In a more recent paper, Scharrer and Schropp (93) have continued their experiments with plants growing in soil. Slight increases in yield due to the follicle

hormone were obtained with soy beans, red clover, and alfalfa. No effect was found upon lupin or corn. Nehring and Möbius (70) have investigated the question of whether the beneficial effects of manure may be due to the latter's content of oestrogenic substances. Numerous samples of "stallmist" showed however an average of only 500 mouse units per kilo. The amount of follicle hormone received by one plant during an ordinary treatment with manure is hence small. More probably the striking growth promoting properties of manure are to be sought in substances such as vitamin B₁ and bios II.

The experiments recorded above, carried out as they were with plants growing under more or less normal conditions, are of interest from a practical standpoint. A more complete elucidation of the rôle of the sex hormones in plant development will probably, however, await a more detailed physiological analysis along lines similar to those applied in the work on vitamin B₁ and biotin. As has already been mentioned, some approach of this kind is now being made.

CONCLUSION

During the past few years the cooperation of physiologist and organic chemist has resulted in the actual isolation of chemical substances which are necessary, in very minute amounts, for the growth of organisms. Such organic compounds, variously known as "vitamins" or "accessory growth factors" are to be distinguished from the ordinary nutrients such as sugar on the one hand and, on the other, from the hormones which are the humoral carriers of correlation. It is also not surprising that these accessory substances, concerned as they are with some of the most vital and most typically "living" functions, possess physiological activity over the entire range of living organisms.

We are now beginning to see, in outline at least, the array of accessory growth factors which, throughout the plant kingdom, are necessary if growth is to take place. Inositol, biotin, vitamins B₁ and B₂, vitamin C, phyto-sterols such as oestrone, now have their places in this array but others must undoubtedly be added. In this brief review it has been impossible to mention all of the morphogenetic effects or all of the growth effects which have been ascribed by various authors to various crude extracts. Beneficial effects of yeast extracts, leaf extracts, or seed extracts, upon seed germina-

tion, pollen tube growth, root growth, stem growth, flowering, etc., have, of course, been reported many times. In the past such effects have been regarded as interesting but obscure, or they have been classified under the vague heading of "stimulation" and dismissed. Each of these "stimulations" will, however, probably be found to have its cause in the action of one, or the interaction of several, of the accessory factors. It should be emphasized however that the task is only begun. The accessory factors themselves are not yet completely known. The ways in which each substance acts remain to be elucidated. And, even more important from a morphogenetic standpoint, the substances thus far investigated appear to be very generally and rather unspecifically necessary for the growth process as a whole. The substances responsible for the direction of growth, for development and differentiation, still offer an almost virgin field.

TABLE 1.

Fungi heterotrophic for vitamin B ₁ .	Fungi autotrophic for vitamin B ₁ .
<i>Phycomyces Blakesleeanus</i>	<i>Mucor</i> sp.
<i>Phycomyces nitens</i>	<i>Zygorhynchus exponens</i>
<i>Chaetocladium macrosporum</i>	<i>Absidia</i> sp.
<i>Parasitella simplex</i>	<i>Rhizopus nigricans</i>
<i>Dicranophora fulva</i>	<i>Thamnidium elegans</i>
	<i>Chaetostylum Fresenii</i>
	<i>Pilaira anomala</i>
	<i>Aspergillus niger</i>

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